Study of *Chrysoperla carnea* larvae digestion rates (Insecta, Planipennia) using immunoassays (ELISAs) (*)

Estudio de la tasa de digestión de larvas de *Chrysoperla carnea* (Insecta, Planipennia) con immunoensayos (ELISAs)

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SUMMARY

Using *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) as predator, and the olive moth *Prays oleae* (Bernard) (Lepidoptera: Plutellidae) as prey, a study of larval digestion rates was carried out by means of enzyme linked immunosorbent assays (ELISAs). A comparison was performed between starved predator larvae and larvae fed irradiated eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) as an alternative prey, having been fed dead *P. oleae* larvae initially. It was found that in the absence of food, digestion was significantly greater, which would ultimately motivate the larvae to actively seek more prey items.

RESUMEN

Se ha realizado un estudio sobre la tasa de digestión larvaria utilizando *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) como depredador y *Prays oleae* (Bernard) (Lepidoptera: Plutellidae) como presa mediante un ensayo de immunoabsorción unido a enzimas (ELISAs). Las larvas depredadoras se alimentaron con larvas muertas de *P. oleae* y posteriormente la mitad se mantuvieron en ayunas y el resto se alimentaron con huevos irradiados de *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae). Se determinó que en ausencia de

alimento la digestión fue significativamente más rápida, lo que podría motivar una mayor actividad hacia la búsqueda de nuevas presas.

INTRODUCTION

Due to the limitations of many techniques in detecting and quantifying predation (Sunderland, 1988), ecologists are increasingly turning to serological methods: chewing predators may consume the entire prey item leaving no external traces; sucking predators leave the exoskeleton, but gut dissections are incapable of differentiating between prey items. The reliance on such physical indicators is obviated with immunoassays.

Enzyme linked immunosorbent assays (ELISAs) have been repeatedly used in the study of predator-prey interactions (Du Devoir & Reeves, 1991; Sopp *et al*., 1992; Buchholz *et al*., 1994; Symondson *et al*., 1997, 1999a, b; Harwood *et al*., 2001). The test is based on the specific reactions between antibodies and the corresponding prey antigens found in the guts of predators. The strength of the final colorimetric reaction is proportional to the amount of target antigen present (Clark & Adams, 1977) and can be measured using a modified spectrometer.

However, the strength of reaction can be influenced by a number of factors, digestion being one of the principal factors. Once a prey has been eaten, digestion takes place. Antisera contain a range of antibodies against different epitopes, some of which will survive longer during the process of digestion (Sunderland, 1988). Both the period of time present in the gut and the digestion rate will affect the meals detectability (Symondson & Liddell, 1993). To complicate matters, different species exhibit different digestion rates (Sopp & Sunderland, 1989; Lövei *et al*., 1990; Symondson & Liddell, 1993) which are in turn influenced by such factors as hunger or conversely the abundance of food (Hance & Rossignol, 1983; Sopp & Sunderland, 1989; Lövei *et al*., 1990), and temperature (Sopp & Sunderland, 1989; Hagler & Cohén, 1990) in which the higher temperature the higher the digestion rate.

To date, the majority of work has centred on adult predators. However, many important natural enemies such as the majority of chrysopids, syrphids and some beetles are only predatory in their larval stages. Given the time constraints placed on their development, it is not known what influences digestion. The object of this study was to see what effects the absence or presence of food subsequent to a prey meal would have on the digestion rate of the larvae of the polyphagous predator, *Chrysoperla carnea* (Stephens, 1836).

MATERIAL & METHODS

C. carnea (Stephens) was chosen as test species given its importance as a biological control agent of the olive moth, *Prays oleae* (Bernard, 1788) (Alrouechdi *et al*., 1981; Campos & Ramos, 1985; Sacchetti, 1990). *C. carnea* is only predatory as larvae since the adults feed on sunflower and corn pollen, and also on flower nectar (Principi and Canard, 1984). A laboratory colony of *C. carnea* was maintained in a controlled temperature chamber at light: dark 18:6 (1500 lux), 24°C ± 5% temperature and 60% ± 5% relative humidity.

All larvae were starved previously for 24 hours to clear the stomachs of previous meals and to potentiate predatory activity since it is known that larvae of *C. carnea* search and attack more readily when hungry (Baumgaertner *et al*., 1981).

Third instar larvae of laboratory reared C. *carnea* were used to determine whether the assay could detect predation and for how long. This instar was chosen since younger larvae were found to die when starved for 24 hours prior to the start of the experiment (T.I. Morris, pers. obs.) and did not take prey larvae so readily. Later instars would pupate early in the absence of food (Principi & Canard, 1984). Similarly when conducting the experiment the larvae would often pupate before the end of the experiment (T.I. Morris, pers.obs.).

Chrysopid larvae were feed with recently defrosted *P. oleae* larvae and allowed to feed for one hour in an Eppendorf tube. The advantage of using Eppendorfs is that it increased the possibility of encounter between predator and prey. Dead larvae were used as it reduced predator handling time and due to the difficulty in obtaining fresh prey from the field at the right time. Those larvae that refused to eat were removed from the experiment.

Ten of the larvae that had fed were frozen at –20°C immediately after feeding, and ten larvae at 12 hour intervals until 48 hours. Half of these were given an excess supply of irradiated eggs of *Ephestia kuehniella* (Zeller, 1878) as an alternative food source. As positive control, *P. oleae* larvae were used as they would give values for maximum protein content. As negative controls, unfed *C. carnea* larvae were used.

All larvae were weighed and stock solutions made in PBS (Phosphate buffered saline). The stock solutions were 1: 20 (w/v). Using disposable pellet pestles (Kontes Glass Co., USA), each specimen was macerated individually in an Eppendorf and the homogenate centrifuged at 8,000 g for 15 minutes. The supernatant was then labelled and stored at -20°C for subsequent assay in an indirect ELISA (Morris, 1997).

To evaluate the effects of feeding and time on protein digestion rate a two-way analysis of variance was performed. Post-hoc comparison for different times after feeding were done using a Bonferroni test.

RESULTS AND DISCUSSION

One of the factors that most influenced the detection of predation in ELISA bioassays is the digestion rate. This was shown using one of the principal olive moth predators, C. *carnea.*

Significant feeding ($F = 7.315$, $p = 0.001$, d.f.= 1) and time ($F = 9.491$, $p = 0.001$, d.f. = 4) effects were observed, whereas the interaction between feeding and time was negligible ($F = 0.681$, $p = 0.609$, d.f. = 4). Initially, immediately after feeding with *P. oleae* larvae, the amount of protein detected in the guts fed and unfed of *C. carnea* larvae was the same (Fig. 1). The amount of protein detected dropped significantly ($p = 0.05$) in starved larvae and feeding larvae between 12h and 24 h (Fig 1). With the passage of time the amount of detected protein diminishes and it is not possible to determine whether this is due to assimilation and/ or digestion (Lövei *et al*., 1990).

As with Fichter & Stephen (1981, 1984), a linear fit best described the antigen decay relationship in our case. Lovei *et al*. (1985), in a re-examination of the previous authors' work found that a logarithmic function improved the fit although in their own work they admitted that a linear regression gave nearly as good a fit. Greenstone & Hunt (1993) attributed the linearity to the use of polyclonal antisera whereby the combined digestion of many antigenic determinants. However the majority of studies using polyclonal antisera showed a negative exponential form of antigen decay (Lövei, *et al*., 1985, 1987; Sopp & Sunderland, 1989; Symondson & Liddell, 1993, 1995).

In this study it was found that digestion was more rapid in starved larvae whereas Lövei, *et al*. (1987) found that the digestion rate of adult carabids, *Bembidion lampros* (Herbst, 1748), increased more rapidly when more food was eaten initially irrespective of whether an alternative prey item was provided subsequently.

Using larvae of the carabid *Poecilus cupreus* L., Lövei *et al*. (1985) found that the digestion rate increased logarithmically with greater periods of prior starvation. However, in a later study a comparison of the effect of subsequent alternative feeding or starvation showed that there were no significant differences between the rates (Lövei *et al*., 1990). Digestion was initially faster in starved predators and then slower resulting in overall longer detection periods (Lövei *et al*., 1990).

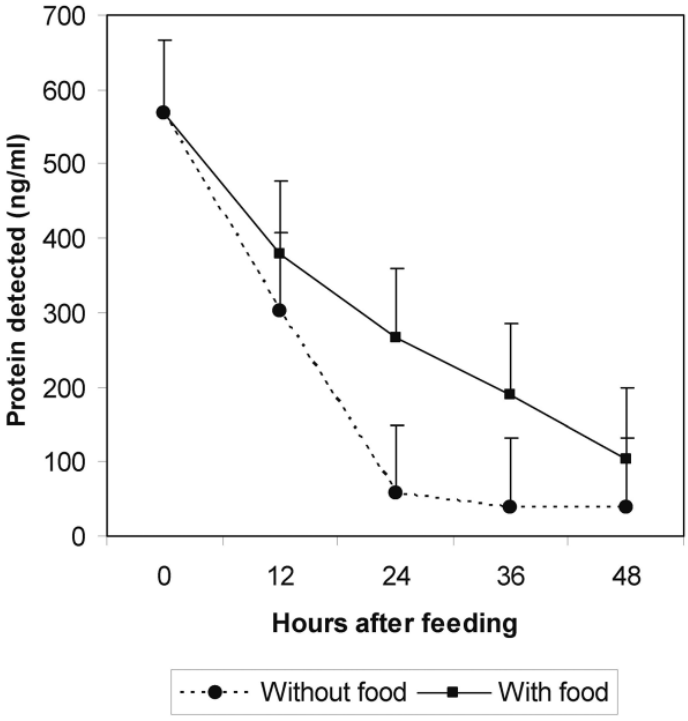

Fig. 1.—*Prays oleae* protein detected in larvae of *C. carnea* maintained with and without food.
Fig. 1.—Proteína de *P. oleae* detectada en larvas de *C. carnea* mantenidas con y sin comida.

Spiders have morphological (gut ramifications) and physiological (low metabolism levels) adaptations to resist low food levels (Riechert, 1992). In this way Fichter & Stephen (1984) could still detect the same proportion of antigen present on day one as on day 12 after being starved. Riechert (1992) considers spiders, even the hunting spiders, on the whole to be sit-and-wait predators. This strategy would explain why spiders have evolved such adaptations.

More active predators such as chrysopid larvae have no such adaptations. In the absence of food, a high digestion rate would induce higher hunger levels, which would in turn spur on searching activity (Baumgaertner *et al*., 1981), at a higher energetic cost (Symondson & Liddell, 1995). Within the developmental framework imposed by time, this is not without its advantages. In the absence of food resources the later instar larvae pu-

pate precociously and the resultant adults emerge deformed and nonviable (T.I. Morris, pers. obs.). Cannibalism is a function of hunger and, although common, it is the nutritionally least preferred option, since it has an overall negative effect on the reproductive capacity (Duelli, 1981; Canard & Duelli, 1984). However survival is greater than with starved individuals (Canard & Duelli, 1984). Fleschner (1950) estimated that for unrestricted development of *C. carnea* larvae, about 10% of 'full ration' is an absolute minimum. The results presented here suggest that hunger is in fact a motivating force for predation driven by a higher digestion rate than when given unlimited access to a food source.

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