Biocontrol of western flower thrips by heteropteran bugs
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The influence of prey density on development, survival and reproduction of *Orius insidiosus*

The influence of the density of eggs of the *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) on development, survival, and reproduction of *Orius insidiosus* (Say) (Heteroptera: Anthocoridae) was investigated. Nymphal survival increased with prey density. Mortality was largely restricted to the first 3 days after hatching. Addition of bean pods to a diet consisting of *E. kuehniella* eggs increased nymphal survival. However, mortality of nymphs was 100% when bean pods were the only food provided. Duration of nymphal development decreased with increasing prey density. The net consumption of prey eggs during nymphal development, and the length of adult hind tibiae increased with prey density. The net consumption rate of adult females increased with prey density until a density of 16 prey eggs/day, whereas the rate of oviposition increased until a density 8 prey eggs/day. The effect of nymphal feeding history on reproduction was investigated in females fed *ad libitum* as adult. Females that developed at a density of 1 prey egg/day had a lower initial ovipositional rate than females that developed when provided ample food. However, total fecundity of both groups was similar, because the ovipositional rate started to decrease earlier in the latter. The correlations between life history traits and prey density can be used adjust food supply in mass cultures to actual requirements.

Predatory bugs in the genus *Orius* are widely used as biological control agents against western flower thrips, *Frankliniella occidentalis* (Pergande), in European greenhouse vegetables. Mass rearing is required to produce adequate numbers. Eggs of *Ephestia kuehniella* Zeller are suitable to rear *Orius* predatory bugs (Chapter 1), but these eggs are quite expensive. Hence, it is important to determine the optimal ratio between input of food and output of predatory bugs.

The rate of increase of a predator population depends on three main components that are affected by the rate at which prey is captured: (1) developmental rate of each instar (2) survival rates within instars, and (3) fecundity of the adults (Beddington *et al*., 1976). In the present study the relation between supply of *E. kuehniella* eggs and each of these component was determined for the predatory bug *Orius insidiosus* (Say). All three components usually increase with prey density to a plateau (Beddington *et al*., 1976). In a mass production system food supply can be set to the level at which these plateaus are reached. However, this is only the first step in improving rearing efficiency. The relation between food supply and life history components can also be used to optimize the ratio of food supply and the production of predatory bugs. This is discussed by Van den Meiracker (Chapter 4).

Nymphal feeding history may affect adult fitness. For the ratio between food input and bug output it may be favourable to keep food supply for nymphs relatively
low. It was investigated if this reduces adult size and fecundity. For production purposes reduced fecundity may be of minor importance, since the major part of the Orius rearing population will be harvested before oviposition starts. However, their efficacy as biocontrol agent may be reduced.

The fraction of the food that is actually consumed was also recorded. How this fraction depends on prey density should also be taken into account in a commercial rearing system. The ratio of food input and bug output may be optimal at a prey density, at which not all prey eggs are consumed. In that case rearing efficiency may be improved by increasing food accessibility.

MATERIALS AND METHODS

Predatory bugs used in the experiments were taken from a laboratory colony. This colony was initiated in 1989 with *O. insidiosus* from Georgia (USA). See Van den Meiracker (1994) for rearing details. All experiments were carried out at 25°C, 75% r.h., and 16L:8D.

Nymphal development, survival, and consumption at different prey densities

Experiments were started in 1991 with first instar nymphs which had hatched 0-3 hours before. They were put individually in 25 ml glass pots with snap caps, lined with filter paper. Ventilation of these pots was provided by a hole in the cap covered with fine nylon gauze. Moisture was provided by a small piece of bean pod, which was replaced every second day. *E. kuehniella* eggs were provided on small pieces of filter paper (25 × 7 mm) in densities of 0, 1, 2, 4, 8, or 16 eggs per piece of filter paper. The initial numbers of nymphs were 48, 36, 24, 20, 16 and 16 at these six densities respectively. Every day the pieces of filter paper with *E. kuehniella* eggs were replaced by new ones, and the predatory bugs were checked for survival or adult eclosion. Only ingested fractions of prey eggs were included in the calculation of net consumption (see Chapter 1). After adult reproduction was recorded (see below), the length of the hind tibia of all males and females was measured.

This experiment was repeated with a small glass test-tube containing wet cotton wool as water source, instead of a piece of bean pod. was used for this purpose. It was replaced every second day. *E. kuehniella* eggs were supplied in densities of 1, 2, 4, or 8 per piece of filter paper. The initial numbers of nymphs were 36, 24, 20, and 16 at these four densities respectively. Survival, developmental times and net consumption were recorded.

Adult consumption and reproduction at different prey densities

After adult eclosion females (from the series with bean pods) were kept at the same prey egg densities, but pieces of bean pod were refreshed every day.

On the first 3 days after adult eclosion, each female was kept with a male of a corresponding series (but without food) for half an hour, or (only on the first day) until mating occurred. In this way females had access to their food during 98% of the day, but mating success was relatively low. Hence, females that did not oviposit were discarded. This resulted in 4, 3, 8, 7, and 5 females at densities of 1, 2, 4, 8, and 16
prey eggs/day. Net consumption and reproduction were recorded 6, 7, and 8 days after adult eclosion. This experiment was repeated with females collected from the stock colony (0-24 hours after adult eclosion) at densities of 0, 1, 2, 4, 8, 16, or 32 prey eggs/day. The number of females per prey density was 10-14. During the first day after adult eclosion these females were kept with a male and allowed to feed ad libitum.

Effect of nymphal feeding history on reproduction

In 1993 newly-hatched nymphs were reared as described above (with bean pods as source of moisture) in two series: (1) with 1 E. kuehniella egg per day (n = 48) and (2) with ample supply of prey eggs (n = 24). After adult eclosion females of both series were each accompanied with a male, while ample supply of prey eggs and a fresh bean pod were provided. Replacement of E. kuehniella eggs and pieces of bean pod, and recording oviposition occurred every second day for a period of 45 days.

Statistical analysis

The Jonckheere test for ordered alternatives (Siegel & Castellan, 1988) was used to test whether or not nymphal developmental times decreased with increasing prey density and whether consumption, nymphal survival, hind tibia length and ovipositional rate increased with prey density. When significant differences were found, medians were separated using the Wilcoxon two-sample test. This test was also used to analyze the influence of moisture source and sex on development and consumption. Differences in nymphal survival between prey densities were tested using the chi-square test. The Fisher exact test was used to analyze the influence of moisture source on survival. Linear regression was applied to determine the correlation between total nymphal consumption and the hind tibia length of adults, and the significance of the correlation coefficient based on the Student's t-distribution was calculated. The same analysis was done for the correlation between tibia length and fecundity. In all tests α was set at 0.05. In the case of multiple comparisons α was corrected to α′ = 1 - (1-α)^1/n (n denotes the number of comparisons). Tests were one-tailed with alternative hypotheses that developmental rate, survival, consumption and ovipositional rate were higher and tibiae were longer (1) at high than at low prey density, (2) with bean than with water, (3) in females than in males.

RESULTS

Nymphal development, survival, and consumption at different prey densities

None of the O. insidiosus nymphs survived until adult eclosion when provided only with a bean pod. Mean longevity on this diet was 2.3 days, but one nymph stayed alive for 11.5 days. Therefore, the density of 0 E. kuehniella eggs/day was excluded from statistical analysis. Mean nymphal survival at the other densities varied from 0.50 to 0.75 (Fig. 1A). Although survival seemed to increase with prey density, this trend was not significant. When free water was available, nymphal survival was
lower (significant only at the density of 1 prey egg/day), and varied from 0.14 to 0.53 (significant difference between prey densities). In the six treatments with bean, 70-100% of nymphal mortality occurred during the first 3 days after hatching. In the four treatments with wet cotton wool this percentage varied from 93% to 100%. Nymphs that died during the first 3 days after hatching, did not consume any prey eggs, with the exception of three nymphs at 1 prey egg/day in the bean series. In all nymphs surviving the first 3 days some (partial) consumption was observed, when prey eggs were available. When only bean pods were present, 13% of the nymphs was still alive after 3 days.

Net consumption rates of nymphs increased stepwise during development, until limited by supply (Fig. 2). During the first days after hatching the net consumption rate was slightly lower in treatments with bean (Fig. 2A) than in treatments with free water (Fig. 2B). On the first day after hatching this difference was significant at densities of 2, 4, and 8 prey eggs/day. The total amount of food consumed during the nymphal stage increased significantly with prey density when beans were present (Fig. 1B). Additionally, females consumed significantly more during the nymphal stage than males at densities of 1, 8, and 16 prey eggs/day. When free water was offered instead of bean pods, total net consumption at 1 prey egg/day increased significantly in both female and male nymphs.

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Figure 1. Influence of the density of *E. kuehniella* eggs on: (A) survival from egg hatching until adult eclosion, (B) pre-imaginal net consumption (± s.d.), (C) duration of nymphal development (± s.d.), and (D) hind tibia length of adults (± s.d.) in *O. insidiosus* (B-D with bean pods present).
INFLUENCE OF PREY DENSITY ON LIFE HISTORY OF ORIUS INSIDIOSUS

Duration of nymphal development decreased significantly with increasing prey density when beans served as a water source (Fig. 1C). Developmental times differed significantly between sexes only when provided with 1 prey egg/day. Providing free water instead of bean pods did not affect developmental time.

The length of the hind tibia of adults increased significantly with daily supply of food during the nymphal stage (Fig. 1D). Hind tibiae were significantly longer in females than in males, except when food supply was 1 prey egg/day. In both females and males a linear correlation was found between total net consumption during the nymphal stage and the length of adult hind tibiae (Fig. 3).

**Adult consumption reproduction at different prey densities**

The net consumption rate of ovipositing O. insidiosus females increased significantly with the density of E. kuehniella eggs in both series (Fig. 4A-B). When the food supply was 8 prey eggs/day or lower, all eggs were usually consumed completely before prey replenishment. In the series with females from the stock colony, median net consumption rates were significantly different between prey densities, except between densities of 16 and 32 prey eggs/day. Differences were not so pronounced in females that were also kept at the specified density during nymphal development, but it should be noted that numbers of tested females were low. At none of the prey densities a significant difference was found between the two series. In males the net consumption rate also increased significantly with prey density (Fig. 4A). Net consumption rates were significantly lower in males than in females at densities of prey 4 eggs/day and higher.
Chapter 2

hind tibia length (mm)

0.65
0.60
0.55
0.50
0.45

0.60
0.55
0.50
0.45

total nymphal consumption of prey eggs

Figure 3. Correlation between pre-imaginal net consumption of E. kuehniella eggs and the hind tibia length of adult O. insidiosus. Linear regression lines: -- females (Y = 0.002931X + 0.4853, R = 0.9124, P < 0.00001), --- males (Y = 0.003244X + 0.4589, R = 0.8797, P < 0.00001).

Figure 4. Net consumption rates (± s.d.) and rates of oviposition (± s.d.) of O. insidiosus at different densities of E. kuehniella eggs during 6-8 days after adult eclosion; dots: females; triangles: males; A+C: adults originating from nymphs developed at specified density; B+D: adults collected from the stock colony.
The rate of oviposition increased significantly with prey density in each of the series (Fig. 4C-D). In females collected from the stock colony, the median rate of oviposition was significantly different between prey densities, except between densities of 8 prey eggs/day and higher. Again, differences were not so pronounced in the other series. Ovipositional rates were not significantly different between the two series, except at the density of 1 prey egg/day, where the ovipositional rate was higher in females from the stock colony. When the food supply was 8 prey eggs/day or lower the ratio between net consumption rate and the rate of oviposition was approximately 1 : 1 (Fig. 5).

**Effect of nymphal feeding history on reproduction**

Low supply of *E. kuehniella* eggs during the nymphal stage of *O. insidiosus* resulted in a longer developmental time, lower nymphal survival and shorter hind tibiae of adult females, when compared with high prey densities (Table 1). This is in agreement with the results presented in Fig. 1. Nymphal feeding history did not affect the preoviposition period, which lasted 3.8 days in both treatments. The initial ovipositional rate was lower in females provided with 1 prey egg/day as nymph than in females that were provided continuously with an ample amount of eggs (Fig. 6). The mean ovipositional rate, 6-8 days after adult eclosion, was 5.4 (s.d. 1.5) in females provided with little food as nymph and 7.7 (s.d. 2.0) in females provided with much food as nymph (significant difference). However, in females that were kept at a low prey density as nymph oviposition started to decrease later, and there was no significant difference in mean fecundity between females kept at low or high prey density as nymph (157.7 and 169.2 eggs respectively).
Table 1. Comparison of developmental time, nymphal survival and female hind tibia length of *O. insidiosus* between two densities of *E. kuehniella* eggs.

<table>
<thead>
<tr>
<th>Pre-imaginal supply of prey eggs</th>
<th>Developm. time nymphs (days ± s.d.)</th>
<th>Nymphal survival(^1)</th>
<th>Hind tibia length adult female (mm ± s.d.)(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females(^1)</td>
<td>Males(^1)</td>
<td></td>
</tr>
<tr>
<td>1 prey egg/day</td>
<td>15.93 ± 1.66</td>
<td>14.07 ± 1.00</td>
<td>0.58</td>
</tr>
<tr>
<td>ample supply</td>
<td>10.13 ± 0.47</td>
<td>8.95 ± 0.47</td>
<td>0.95</td>
</tr>
</tbody>
</table>

\(^1\) Significant difference between supply rates.

Figure 6. Ovipositional rates of *O. insidiosus* developed at two prey densities.

**DISCUSSION**

**Nymphal development and consumption at different prey densities**

When the supply of *E. kuehniella* eggs was reduced, the developmental rate of *O. insidiosus* nymphs decreased considerably, but even at the lowest density (1 prey egg/day) development could be completed. Nymphal survival increased significantly with prey density when free water was provided, but not when bean pods were present. It is striking that nearly all mortality occurred during the first 3 days after hatching, when food requirements still are relatively low. Some consumption appeared to be essential for survival during the first days after hatching. Survival was probably not limited by the amount of prey eggs provided per day, but rather the chance to find them. This is corroborated by an experiment in which a group of 15 *O. insidiosus* nymphs, provided with 15 *E. kuehniella* eggs/day, was compared with nymphs kept individually with 1 prey egg/day (Chapter 3). Thus, although food supply per nymph was equal, the chance to find a single prey egg was greater in
groups. Three days after hatching, survival in groups was 0.92, whereas net consumption was 95-100%. Survival of nymphs kept individually was only 0.33, whereas net consumption was only 17-30%.

When food supply of \textit{O. insidiosus} was reduced to 1 \textit{E. kuehniella} egg/day, nymphs were still able to complete their development. Their developmental rate decreased by more than 30\%, probably to consume still enough prey eggs to attain maturity. Such flexibility is not typical for all predacious Heteroptera. At prey densities, high enough to complete development, the developmental rate of \textit{Notonecta undulata} Say was hardly affected by prey density (Toth & Chew, 1972). Instar duration of \textit{Blepharidopterus angulatus} (Fallén) was not affected by prey density (Glen, 1973). Contrary to \textit{O. insidiosus}, the minimal daily food supply to survive to the next instar, increased with the consecutive instars of \textit{N. undulata} and \textit{B. angulatus} (Toth & Chew, 1972; Glen, 1973). Additionally, Hollingsworth & Bishop (1982) did not find an effect of late-instar \textit{Myzus persicae} (Sulzer) density on developmental time of \textit{Orius tristicolor} (White). However, at lower prey densities than tested, such an effect may occur in \textit{O. tristicolor}, or survival to the adult stage may be reduced to zero.

The amount of food consumed during nymphal development decreased drastically with prey density. Nymphs of \textit{O. insidiosus} were able to reach the adult stage consuming only one quarter of the amount of food consumed by nymphs kept with ample supply of prey eggs (\textit{cf.} Chapter 1). Balduf (1950) found that \textit{Sinea diadema} (F.) females and males could complete their development on a fifth and a sixth of their maximal food consumption, respectively. The ability to complete development on such low amounts of food may contribute to the efficacy of \textit{O. insidiosus} as a biocontrol agent. It increases the chance that the predator population persist at times of prey scarcity.

\textbf{Role of plant feeding}

Addition of bean pods to a diet consisting of eggs of \textit{E. kuehniella} increased survival of \textit{O. insidiosus} nymphs, but did not accelerate their rate of development. Just the reverse was found by Bush et al. (1993) who added green beans to diets consisting of aphids or eggs of \textit{Heliothis virescens} (F.). This did not affect nymphal survival of \textit{O. insidiosus}, but it shortened nymphal development. Kiman & Yeargan (1985) who also supplemented \textit{H. virescens} eggs with bean pods as food for \textit{O. insidiosus} found an effect neither on nymphal survival nor on rate of development. The effect of additional plant feeding on fecundity and adult longevity has not been studied in \textit{Orius} predatory bugs, since plant material is here mostly used as a substrate for oviposition. However, inclusion of green beans to the nymphal diet may also increase fecundity, as shown for \textit{O. insidiosus} by Bush et al. (1993). Kiman & Yeargan (1985), who did a similar experiment, did not find such an effect. Armer et al. (1998) showed that \textit{O. insidiosus} fed on xylem contents of soybean, providing them water, and probably on mesophyll, which may provide some nutrients (starches, sugars, amino acids). Usually \textit{Orius} predatory bugs are not able to develop entirely feeding on green parts of plants, \textit{e.g.} \textit{O. insidiosus} (this study; Kiman & Yeargan, 1985), \textit{O. vicinus} (Ribaut) (Fauvel, 1971), and \textit{O. tantilus} (Motschulsky) (Mituda & Calilung). Nevertheless, complete development on bean pods has been
reported for *O. tristicolor* (Salas-Aguilar & Ehler, 1977) and *O. insidiosus* (Richards & Schmidt, 1996).

In summary, it can be concluded that addition of plant food to rearing diets of *Orius* may have favourable effects on several life history traits. In mass cultures, providing additional plant food may be a relatively inexpensive way to increase survival and reproduction. Even if they would only serve as source of moisture, the use bean pods may be more convenient than supplying water. Replacing the bean pod from which the nymphs hatch by a fresh one has to be done only once during nymphal development (Van den Meiracker, unpubl.). In the field plant feeding may support the persistence of *Orius* populations in times of prey scarcity, as feeding on pollen probably does (Van den Meiracker & Ramakers, 1991; Van de Veire & Degheele, 1993).

**Adult consumption and reproduction at different prey densities**

The ovipositional rate of *O. insidiosus* increased with prey density, until a plateau was reached. Such relationships between prey density and fecundity are commonly found in arthropod predators (Beddington *et al.*, 1976), and likewise in several other predacious Heteroptera, e.g. *Anthocoris confusus* Reuter (Evans, 1976), and *Podisus maculiventris* (Wiedenmann & O'Neil, 1990; Legaspi & O'Neil 1993). In a simple model Beddington *et al.* (1976) described the relationship between ingested food and fecundity. They assumed that below a certain threshold all the ingested food was allocated to maintenance metabolism, and that the surplus energy above this threshold is linearly related with fecundity. This may be true for *O. insidiosus*, but there are two deviations from the general pattern. First, there is some oviposition by females without *E. kuehniella* eggs, that were well fed as nymph (Fig. 5B). This may be due to a 'carry over' from earlier stages (Beddington *et al.*, 1976), but it may also be due to the fact that these females were fed *ad libitum* during the first day after adult eclosion. Second, the ovipositional rate does not increase as fast as the net consumption rate at the highest prey densities. This may indicate accumulation of reserves or less efficient conversion at high ingestion levels.

**Effect of nymphal feeding history on adult size and reproduction**

When prey supply was reduced, nymphal survival and the rate of development of *O. insidiosus* decreased. In a mass-rearing system these decreases may be outweighed by the savings on expenses of food. However, if nymphal feeding history influences the quality of adults, mass-rearing efficiency as well as their efficacy as biocontrol agents may be affected. A linear correlation was found between pre-imaginal net consumption by *O. insidiosus* and prey egg supply. Reproduction may also be influenced by nutritional history, since fecundity is often related to size (Beddington *et al.*, 1976). Indeed, the initial ovipositional rate of *O. insidiosus* was reduced when nymphal food supply was reduced. Evans (1982) reared *P. maculiventris* females of different size by varying food supply in the fifth instar, and found a strong correlation between size and rate of oviposition. In both studies limited food supply may have reduced the number of ovarioles, (as observed in several insects, see Engelmann, 1970), and thereby the maximal ovipositional rate.
Consequences for mass rearing

Eight eggs of *E. kuehniella* per day seems to be the best feeding regimen for both nymphal development and adult reproduction. Nymphal survival, developmental rate, and adult size did not further increase at higher prey densities, and it was also the highest prey density at which nearly all provided prey eggs were consumed completely by adult females. Moreover, the ratio between net consumption and oviposition became less favourable at higher prey densities. However, for commercial purposes it may be economic to rear at lower prey densities during certain periods. In the first part of the nymphal stage food supply can be lower than 8 prey eggs/day per nymph, because food requirements are lower at that stage. The net consumption rate of males reached a plateau at 3 prey eggs/days. Thus, when the sex ratio in the culture is 1:1, providing 5.5 prey eggs/day per adult individual will suffice. Furthermore, low food supply during nymphal development only has a relatively small effect on the ovipositional rate.

Although a supply of 1 prey/day during the entire nymphal stage reduced nymphal survival and the rate of oviposition, this feeding regimen may still be economic for mass rearing. Van den Meiracker found that nymphal survival was not reduced, during the first 3 days after hatching, when nymphs were kept in groups at this feeding regimen (Chapter 2). The consequences of the reduced ovipositional rate for mass rearing are limited, since the major part of the rearing population will be harvested before oviposition starts. Furthermore, the savings on food expenses are so high that some losses can be tolerated (Chapter 4). On the contrary, the effect of reduced nymphal food supply on efficacy of *O. insidiosus* as biocontrol agent gives rise to more concern. A decreased ovipositional rate released females can be compensated by increasing introduction rates, if the savings on expenses of food are high enough. However, the effect of reduced nymphal food supply on e.g. mobility, searching capacity, capture success, and resistance to starvation in the greenhouse should also be investigated.

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