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ORIGINAL ARTICLE

Intraguild predation of *Orius niger* (Hemiptera: Anthocoridae) on *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae)

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Abstract. Intraguild predation of a generalist predator, *Orius niger* Wolff (Hemiptera: Anthocoridae) on *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae), was determined in choice and no-choice experiments using a factitious host, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), under laboratory conditions. Choice and no-choice experiments were conducted in order to assess the level of intraguild predation of *O. niger* on *E. kuehniella* eggs parasitized by *T. evanescens*. In no-choice experiments, approximately 50 sterile (1) non-parasitized, (2) 3-day-old parasitized, or (3) 6-day-old parasitized *E. kuehniella* eggs were offered to 24-h-old females of *O. niger* in glass tubes. In choice experiments approximately 25 eggs of two of the three groups mentioned above were offered to 24-h-old *O. niger* females. In both choice and no-choice experiments, *O. niger* consumed more non-parasitized eggs of *E. kuehniella*. However, intraguild predation occurred, especially of 3-day-old parasitoids, but very few 6-day-old parasitized eggs were consumed. The preference index was nearly 1 indicating *O. niger* preferred mainly non-parasitized *E. kuehniella* eggs. A lower level of intraguild predation is expected under field conditions but needs to be investigated using further experiments.

INTRODUCTION

Interspecific interactions in insects could be classified as either (1) competition, (2) predation/parasitism, (3) mutualism, (4) commensalism or (5) amensalism (Polis et al., 1989). Intraguild predation is a combination of competition and predation/parasitism and is defined as the killing or eating of species that use similar limiting resources (prey) (Polis et al., 1989). Intraguild predation is common in nature when species compete for a shared (extraguild) prey in the same ecosystem (Polis et al., 1989; Holt & Polis, 1997). This situation affects the distribution, abundance and evolution of many species in natural and controlled ecosystems (Polis et al., 1989). The use of more than one natural enemy in biological control programmes can lead to direct and indirect interactions between intraguild predators (IGP) or/and competition (Janssen et al., 1999). These interactions may limit the efficiency of biological control agents against pest species (Yano, 2005).

In the order Lepidoptera there are a large number of economically important species of pests such as the Cotton bollworm (*Helicoverpa armigera* Hübner, Lepidoptera: Noctuidae) and Tomato pinworm (*Tuta absoluta* Meyrick, Lepidoptera: Gelechidae), which occur in both field and protected vegetable production areas. Several of the biological control strategies against lepidopteran species of pests in the field use egg parasitoids (Smith, 1996; Consoli et al., 2010). The trichogrammatid parasitoids are the most common and most effective natural enemy of Lepidopteran pests (Özpınar & Kornoşor, 1994; Smith, 1996; Consoli et al., 2010). Although they are very effective natural enemies, their efficiency might be affected by several ecological interactions such as intraguild predation. Anthocorids (Hemiptera: Anthocoridae) are important predators of lepidopteran eggs (Pericart, 1972; Önder, 1982) and may reduce the efficiency of *Trichogramma* parasitoids (Lingren & Wolfenbarger, 1976). *Orius* Wolff is the most important genus of this family that feed upon lepidopteran eggs (Sansone & Smith, 2001) and are commonly used in biological control programmes against lepidopteran pests on cultivated plants (Péricart, 1972). Some studies have shown the occurrence of intraguild predation by generalist predators on eggs parasitized by *Trichogramma* (Brower & Press, 1988; Kuhar et al., 2002) and in some cases the predators prefer non-parasitized eggs (Alrouechdi & Voegele, 1981; Ruberson & Kring, 1991). However, determination of intraguild predation on parasitoids in natural conditions can be difficult. For this reason, it is better to determine if intraguild predation occurs in the laboratory before undertaking field experiments.

The Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyrallidae) is important since its eggs and larvae are commonly used for mass rearing of many natural enemies (such as *Orius* spp., *Macrolophus* spp., *Trichogramma* spp.), which are released in many biological control programmes against many species of pests. The members of the Genus *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) are well-known egg parasitoids of many insect pests, which mainly belong to the order Lepidoptera (Smith, 1996; Oztemiz, 2007). Trichogammatid egg parasitoids are reared on sterilized eggs of the factitious host, *E. kuehniella* in the laboratory or in mass rearing conditions.

In this study, we planned a series of experiments to determine the intraguild interactions between *Orius* and Trichogrammatids using *Ephestia kuehniella* eggs as the laboratory target insect. The aim of the present work was to determine the feeding behaviour of *O. niger* when presented with eggs of *E. kuehniella* that are either parasitized or not parasitized by *T. evanescens* in no-choice and choice experiments.

MATERIAL AND METHODS

Ephestia kuehniella rearing

The colony of *E. kuehniella* came from the Biological Control Research Institute at Adana, Turkey. The colony was reared in the Entomology Laboratory at the University of Çukurova, Faculty of Agriculture, Department of Plant Protection, Adana, Turkey for two years. *E. kuehniella* was reared on a wheat flour : corn flour (1 : 1) mixture, in climatic rooms at 25°C, 60% relative humidity in the dark. Thousand eggs of *E. kuehniella* were added to each 100 g food material.

Orius niger rearing

The predatory bug, *Orius niger* was collected from cotton flowers at Adana, Turkey. The stock culture was reared on sterilized eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) in the laboratory for six months. *Orius* individuals were reared in incubators at 25°C, 60% relative humidity under a 16L : 8D photoperiod. Nylon plastic cups (0.5 l capacity) with two ventilation holes covered with fine mesh muslin were used as rearing units. Fresh bean pods were used as an oviposition substrate and *E. kuehniella* sterilized eggs glued onto blue cards were provided as food for *O. niger*. Adults were kept in other rearing cages to prevent cannibalism. Bean pods with *Orius* eggs were removed from the adult units three times per week and were placed in other cups for pre-imaginal rearing.

Trichogramma evanescens rearing

The colony of *T. evanescens* was supplied by the Biological Control Research Institute at Adana, Turkey and reared in the laboratory. *T. evanescens* was reared on one-day-old *E. kuehniella* eggs, which were previously sterilized using ultraviolet light, in climatic chambers at 25°C, 70% relative humidity under a 14L : 10L photoperiod. The sterilized eggs of *E. kuehniella* were glued with 10% Arabic gum to paper strips (1 × 8 cm). The paper strips were placed in glass tubes (2.5 × 16 cm) with 50% honey-water solution that was provided in film form as food for the *T. evanescens*. Parasitized eggs of *E. kuehniella* were removed from the glass tubes every 8 days, before the emergence of parasitoids and placed and reared in other tubes.

Experimental design

No-choice and choice experiments were carried out in order to determine if *O. niger* fed on *Ephestia kuehniella* eggs that were parasitized by *T. evanescens* at different stages of development;

(i) non-parasitized eggs,
(ii) 3-day-old parasitized eggs (yellow) and
(iii) 6-day-old parasitized eggs (black) by *T. evanescens*.

Approximately 50 one-day-old sterilized eggs of *E. kuehniella* were glued on blue cards (3 × 1 cm), which when they were 3 and 6 days old, respectively, were exposed for 24 h to attack by *T. evanesces,* which provided the material for the above experiments (ii, iii).

Females of *O. niger* (< 24-h-old) that were starved for 24 h were used in each experiment.

No-choice experiments

Only one type of host eggs was provided to each female of *O. niger* in a glass tube (8 × 1.5 cm) plugged with cotton for 24 h. The number of *E. kuehniella* eggs consumed by *O. niger* were counted under a stereo binocular microscope (× 40 magnifications) (Euromex Nexius Zoom). A randomized design was used in these experiments, with one factor (at three levels) and at least 12 replications per treatment. All experiments were conducted in an incubator at 25°C, 60% relative humidity under a 16L : 8D photoperiod.

Choice experiments

Three different eggs combinations were used in these experiments:

(i) 25 non-parasitized eggs vs. 25 parasitized eggs (3-day-old);
(ii) 25 non-parasitized eggs vs. 25 parasitized eggs (6-day-old) and
(iii) 25 parasitized eggs (3-day-old) vs. 25 parasitized eggs (6-day-old).

Each combination of eggs were presented to a single female of *O. niger* for 24 h in a glass tube (8 × 1.5 cm) plugged with cotton. The level of consumption and preferences of *O. niger* for eggs of *E. kuehniella* that were parasitized by *T. evanescens* were determined by examining the eggs under a stereo binocular microscope. A randomized design was used in these experiments, with one factor (at three levels) and at least 12 replications per treatment. All experiments were conducted in a climatic chamber at 25°C, 60% relative humidity under a 16L : 8D photoperiod.

Statistical analyses

In the no-choice experiments, the number of eggs consumed in each group was compared using Duncan's test at a $P < 0.05$ significance level after first subjecting them to an analysis of variance (ANOVA). The paired t-test was used in binary comparisons in the choice experiments with a confidence level $P < 0.05$. In the choice experiments, total numbers of eggs consumed in each combination were also compared using Duncan's test at a $P < 0.05$ significance level after first subjecting them to an ANOVA. We used Manly's preference index (Manly, 1974) for each egg combination, i.e., non-parasitized and 3-day-old parasitized eggs

$$\beta_1 = \frac{\log\left(e_1 / A_1\right)}{\log\left(e_1 / A_1\right) + \log\left(e_2 / A_2\right)}$$

where β_1 is the preference for egg type 1, A_1 and A_2 are the numbers of eggs of types 1 and 2 offered. And e_1 and e_2 are the numbers of eggs remaining at the end of the experiment. The preference index (β_1) can be between 0 and 1. β-values higher than 0.5 indicate a preference for egg type 1. IBM SPSS 21 (SPSS, 2012) statistical package software was used for paired t-tests and

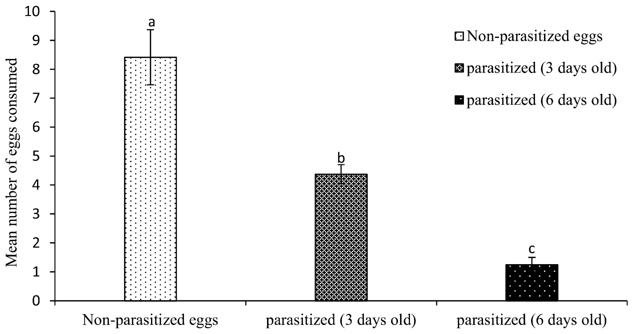

Fig. 1. Mean number (± SEM) of non-parasitized, 3 day old parasitized and 6 day old parasitized eggs of *E. kuehniella* eaten in 24 h by a female *O. niger* in no-choice experiments. Differences between columns with different letters are statistically significant according to Duncan's test (P < 0.05).

ANOVAs (SPSS, 2012). For Manly's preference index MS Excel 2010 was used.

RESULTS

No-choice experiment

Orius niger consumed more non-parasitized *E. kuehniella* eggs than both yellow and black parasitized *E. kuehniella* eggs in no-choice experiments. This predator consumed 8.4 non-parasitized eggs, 4.33 3-day old parasitized eggs (yellow) and 1.08 6-day old parasitized eggs (black) (Fig. 1). Mean numbers of eggs consumed in the different experiments were significantly different ($F_{(2,21)} = 36.124$, $p < 0.0001$).

Choice experiment

In choice experiments, two combinations of types of eggs of *E. kuehniella* were offered at the same time to females of *O. niger*. The predator preferred non-parasitized eggs (t = 6.021, df = 22, $p < 0.0001$) and 3-day-old parasitized eggs (t = 7.070, df = 22, $p < 0.0001$) to 6-day old parasitized eggs. When non-parasitized eggs and 3-day-old parasitized eggs were provided at the same time, *O. niger* significantly preferred the non-parasitized eggs (t = 4.655, df = 22, $p < 0.0001$; Table 1). The preference indices varied between 0.95–0.98 when non-parasitized eggs were provided. The 3-day-old parasitized eggs were preferred to 6-day-old parasitized eggs, with a preference index of 0.99 ± 0.01 (Table 2).

The total numbers of eggs consumed was statistically highest in tubes with non-parasitized eggs and 3-day-old parasitized eggs (yellow) ($F_{(3,40)} = 5.097$, $p < 0.004$; Fig. 2). In the absence of non-parasitized eggs fewer eggs were consumed.

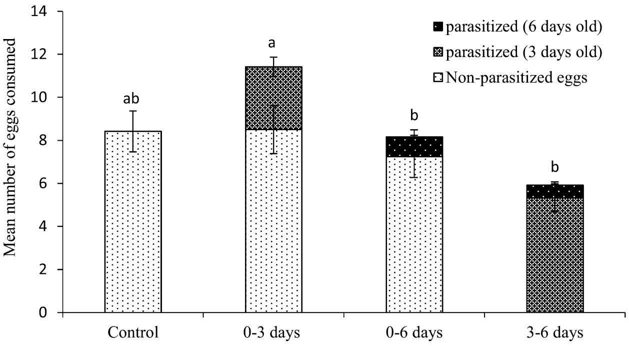

Fig. 2. Mean numbers (± SEM) of eggs consumed by a female of *O. niger* when non-parasitized eggs (control) alone were provided and when non-parasitized eggs and 3 day old parasitized eggs (0–3 days), non-parasitized eggs and 6 day old parasitized eggs (0–6 days), and 3 day old parasitized eggs and 6 day old parasitized eggs (3–6 days) of *E. kuehniella* were provided. Differences between columns with same letters are not statistically significant according to Duncan's test ($p < 0.05$).

DISCUSSION

Like other generalist predators, species of *Orius* may occur at the same time as species of *Trichogramma* in agroecosystems and often share one or more species of prey/host in common (Kayapınar, 1991; Romeis & Shanower, 1996; Tillman et al., 2009; Zappala et al., 2013). Results of this study reveal the occurrence of intraguild predation by *O. niger* on eggs of *E. kuehniella*, which were previously parasitized by *T. evanescens*. On the other hand, based on the results of both the choice and no-choice experiments, it is obvious that *O. niger* avoids consuming black parasitized eggs of *E. kuehniella*.

Previous studies mostly show a similar pattern. It is reported in many studies that anthocorid, pentatomid and mirid predators consume few parasitized eggs that contain a pupa of the *Trichogramma* parasitoid (Brower & Press, 1988; Oliveira et al., 2004; Chailleux et al., 2013; Cabello et al., 2015). It is reported that there is little intraguild predation by the hemipteran predators *Nesidiocoris tenuis* (Reuter) on *Trichogramma achaeae* Nagaraja & Nagarkatti (Cabello et al., 2015); *Macrolophus pygmaeus* Rambur on the same species of parasitoid (Chailleux et al., 2013) and *Podisus maculiventris* (Hemiptera: Pentatomidae) on *Trichogramma brassicae* Bezdenko (Oliviera et al., 2004), especially of black eggs. During the pupation of trichogrammatids, melanin is deposited within the host's egg, which darkens the colour of the egg. This material increases the mechanical resistance of the egg shell, thus

Table 1. Mean numbers (± SEM) of non-parasitized (0), 3-day-old parasitized (3) and 6-day-old parasitized (6) eggs of *E. kuehniella* consumed in 24 h by a female *O. niger* in choice experiments (Means followed by "*" are significantly different based on paired t tests at a significance level of 0.05).

Groups	Choices	Mean ± SEM	F	t	P
Non-parasitized (0) vs. 3-day-old parasitized (3)	0 3	8.5 ± 1.11* 2.9 ± 0.45	21.667	5.366	< 0.0001
Non-parasitized (0) vs. 6-day-old parasitized (6)	0 6	7.3 ± 0.99* 0.9 ± 0.34	36.983	6.008	< 0.0001
3-day-old parasitized (3) vs. 6-day-old parasitized (6)	3 6	5.3 ± 0.66* 0.6 ± 0.15	49.985	6.705	< 0.0001

Table 2. Mean numbers (± SEM) of eggs consumed and mean preference indices (± SEM) of *O. niger* female when offered different combinations of different kinds of eggs.

Egg combinations		Consumed eggs		Preference index	n
Non-parasitized	3-day-old parasitized	8.5 ± 1.11	2.9 ± 0.45	0.95 ± 0.01	12
Non-parasitized	6-day-old parasitized	7.3 ± 0.99	0.9 ± 0.34	0.98 ± 0.01	12
3-day-old parasitized	6-day-old parasitized	5.3 ± 0.66	0.6 ± 0.15	0.99 ± 0.01	12

protecting the parasitoid from natural enemies (Pintureau et al., 1999).

Another result of this study, is the significant difference in the number of yellow parasitized and non-parasitized eggs consumed, with a marked preference for non-parasitized eggs. Oliviera et al. (2004) and Cabello et al. (2015) report the same preference. Chailleux et al. (2013), contrary to our results with *O. niger*, reveal that *M. pygmaeus* does not show any preference when feeding on yellow parasitized and non-parasitized host eggs. Even if the colour does not change visibly, the content of the eggs changes during the first three days of parasitization. During egg laying, *Trichogramma* females inject a venom into the egg of its host, which digests the contents of the egg (Knutson, 1998). Parasitoid eggs hatch in about 24 h and the larvae start feeding on the liquid and solid contents of host's egg. All the contents are consumed by the larva within 8–10 h of the parasitoid's egg hatching during which time the larva increases 40-fold in size (Wu et al., 2000). Hence, feeding by predators on a parasitized egg, even if it is yellow, would be more difficult than feeding on a non-parasitized egg. Small body size (2 mm) and relatively weak mouthparts could make it harder for *O. niger* to eat 2–3 days old parasitized eggs, which are still yellow in colour.

On the other hand, some studies report that both chrysopids and coccinellids consume equal numbers of parasitized and non-parasitized eggs (Alrouechdi & Voegele, 1981; Roger et al., 2001). Most of the predators that do not discriminate between parasitized and non-parasitized eggs have chewing mouth parts, which would make it easier to overcome hardened egg shells.

As an exceptional result, Lingren & Wolfenbarger (1976), report equal consumption of black parasitized and non-parasitized eggs of *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) by *Orius insidiosus* Say. However, this report is based on field observations of a few eggs (n = 23).

Rosenheim & Harmon (2006) suggest that omnivorous intraguild predators are more likely to consume intermediate predators than coincidental intraguild predators, thus the risk of intraguild predation is lower for coincidental intraguild predators. According to Polis et al. (1989)'s definition, intraguild predation of *O. niger* on *T. evanescens* is coincidental intraguild predation and since *O. niger* consumes fewer parasitized *E. kuehnilla* eggs than non-parasitized eggs, our results are in accordance with Rosenheim & Harmon (2006)'s suggestion.

Similar studies that have been done in microcosms (Chailleux et al., 2013) and in greenhouse conditions (Cabello et al., 2015) show a reduced percentage of intraguild predation due to reduced encounter rates. Taking this into account, it is expected that the degree of intraguild predation of *O. niger* on *T. evanescens* is negligible but this still needs to be supported by field trials.

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