Oviposition behaviour of the mirid *Macrolophus pygmaeus* under risk of intraguild predation and cannibalism

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Abstract

Zoophytophagous mirid species, that feed and develop either on prey or plant resources, are often found simultaneously on the same host. Hence, these species can engage in both intraguild predation and cannibalism, which can pose a threat to mirid eggs. Ovipositing females may respond to such
risks of predation on their eggs by reducing the number of eggs laid or selecting safer oviposition sites. We tested the oviposition behaviour of *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) females under the risk of cannibalism by *M. pygmaeus* males and intraguild predation by *Nesidiocoris tenuis* (Reuter) males (Hemiptera: Miridae) under laboratory conditions. Intraguild predators and cannibals were introduced during or after the oviposition period. The number of eggs laid (using counts of newly hatched nymphs) and their proportion on each part of a tomato plant were both measured. The results reveal that only cannibalism by *M. pygmaeus* males after the period of oviposition significantly decreased the number of hatched eggs. Cannibalism thus represents a greater risk to mirid eggs than intraguild predation. The *M. pygmaeus* female responded to the presence of potential intraguild predators (or competitors) by decreasing the number of eggs laid in the upper leaves. The results suggest that *M. pygmaeus* females avoid competition by *N. tenuis*, by laying fewer eggs on upper leaves. Cannibalism could regulate zoophytophagous predator populations under prey scarcity conditions and minimize the risk of crop damage associated with those biological control agents.

**Key words** anti-predator behaviour; cannibalism; intraguild predation; *Macrolophus pygmaeus*; *Nesidiocoris tenuis*; zoophytophagy.

**Introduction**

Zoophytophagous mirids have the ability to substitute zoophagy for phytophagy to complete their development and reproduction (Wiedenmann *et al.*, 1996; Lucas & Rosenheim, 2011). These omnivorous predators can survive in agricultural system during prey shortage and overcome rapid spatial and temporal changes in prey availability (Coll & Guershon, 2002). Several mirid species exploit a common prey and host plant simultaneously (Sanford, 1964; Castañé *et al.*, 2004, Naselli *et al.*, 2017). Hence intraguild predation (IGP: predation on an interspecific competitor) and cannibalism are observed among those predators (Lucas & Alomar, 2001, Moreno-Ripoll *et al.*, 2012; Hamdi *et al.*,...
2013; Perdikis et al., 2014), and can induce anti-predator behaviour as a response to the potential risk. The mirids lay eggs in their host plant tissue, expectedly giving them protection against predators (Sanford, 1964; Wheeler, 2001). However, such a strategy might be less effective against mirid predators (both intraguild predators and cannibals) as these have piercing-suckling mouthparts that allow them to exploit eggs embedded in the tissue. Thus the perceived risk of intraguild predation and cannibalism on mirid eggs could influence the oviposition behaviour of females, and therefore the effectiveness of zoophytophagous mirids as biological control agents against other mirids (Blaustein, 1999).

Several organisms alter their oviposition behaviour in response to the perceived risk of predation (Blaustein, 1999; Rieger et al., 2004; Fontaine & Martin, 2006). On a short temporal scale, risk-sensitive oviposition would lead to quantitative changes (e.g. delaying oviposition in presence of risk of predation) (Montserrat et al., 2007; Carrasco & Kaitala, 2009; Walzer & Schausberger, 2011; Velasco-Hernández et al., 2013). For instance, Montserrat et al. (2007) observed that females of the phytoseiid mite *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae) are able to retain their eggs when exposed to egg predation risk, which allows females to reach safer sites to oviposit. On longer term, the female's response to the risk of predation would generate a qualitative change in the oviposition behaviour in such a way that eggs should be laid in safer sites (Stav et al., 1999; Angelon & Petranka, 2002; Blaustein et al., 2004; Choh et al., 2010). Hence, the distribution of eggs should vary depending on the risk of predation. Mirid females could prefer alternative hosts or use different parts of their usual host.

Intraguild predation and cannibalism are different threats and, hence, may generate varying behavioural responses by ovipositing females. Schellhorn and Andow (1999) found that both *Adalia bipunctata* (Linnaeus) and *Coleomegilla maculata* (De Geer) (Coleoptera: Coccinellidae) suffered more from cannibalism on eggs than from intraguild predation. Authors suggested that cannibalism is more frequent than intraguild predation because *A. bipunctata* and *C. maculata* use different part of
the plant for oviposition (top and bottom of the plant respectively). Therefore, the encounter rate with their conspecific eggs is higher than with their competitor eggs. The effect of the risk of intraguild predation and cannibalism on oviposition site selection by mirid females could be rather different. Females may gather their eggs in a limited space in response to the presence of intraguild predators (assuming both species do not use space identically), which would lead to egg aggregation. In contrast, females could use various and unusual oviposition sites under risk of cannibalism. Thus, eggs would be more scattered when cannibals are a potential threat (e.g. when alternative food resources are scarce) (Cottrell & Yeargan, 1998; Laycock et al., 2006; Hamdi et al., 2013).

In this study, we test the hypothesis that *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) (formerly sold as *M. caliginosus*) (hereafter referred to MP) females are sensitive to both intraguild predation risk by *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) (hereafter NT) and cannibalism risk on their eggs. Both zoophytophagous mirids spontaneously colonize tomato plants or are released as biological control agents, and engage in intraguild interactions (Lucas & Alomar, 2001; Lucas & Rosenheim, 2011; Perdikis et al., 2014), including cannibalism (Moreno-Ripoll et al., 2012; Hamdi et al., 2013). The simultaneous presence of these predators can enhance biological control of whiteflies *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Urbaneja et al., 2009; Urbaneja et al., 2012; Biondi et al., 2018).

*Macrolophus pygmaeus* contrasts with NT by using the middle and lower leaves of the tomato plants to feed and lay eggs, but not the upper stratum of the plants as done by NT (Castañé et al., 2011; Perdikis et al., 2014). Therefore, on this part of the plant, MP would have higher encounter rate with their conspecific eggs than the intraguild predator NT. We predict that: (i) risk of cannibalism will have a greater impact on the quantity of eggs laid by MP females than the risk of intraguild predation; (ii) risk of cannibalism will force MP females to oviposit eggs on the upper part of their host plant, whereas under the risk of intraguild predation eggs would aggregate in the lower part of the plant.
Materials and methods

Mirid populations and rearing conditions

*Macrolophus pygmaeus* and *NT* individuals were both reared at IRTA facilities (Cabrils, Catalunya, Spain) (Agustí & Gabarra, 2009). Both mirids population are reared on tobacco plants (*Nicotiana tabacum* L.) and fed on a diet of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs. Both colonies originated from local tomato fields and are refreshed annually.

Population were kept in acrylic cages in climatic chambers at 25°C, 70% RH, 16 : 8 L : D regime. *Macrolophus pygmaeus* females between one and two weeks of age were randomly assigned to each treatment.

Laboratory experiments

The oviposition behaviour of MP females under risk of intraguild predation on eggs by NT males and of cannibalism by MP males was studied on ~160 mm tomato plants, *Lycopersicon esculentum* (Miller) (Solanaceae), placed in cages made from micro-perforated plastic bags (100 mm diameter by 200 mm height). The host plants used for tests were prepared to leave only five leaves on the plant. All exceeding leaflets (upper leaves) were removed. No additional food was provided as the availability of quality food resources can limit both intraguild predation (Perdikis et al., 2014) and cannibalism (Hamdi et al., 2013).

In all trials, four fertilized MP females were released in each cage and allowed to lay eggs for four days. These females were then removed. The use of four MP females aimed to avoid great variability inherent to only use one female per cage. The potential effect of competition among females or cannibalism was the same for all treatments. Additionally, MP or NT males were also added into the cages, at the same times as females or afterwards to (a) determine the IGP or cannibalism by males, and (b) evaluate the effect of cannibalism or IGP and changes in oviposition behaviour in response to
predation risks. Hence, MP females were exposed to the following treatments: (i) Cannibalism during oviposition (DO CNB): four MP males introduced together with the females; (ii) Intraguild predation during oviposition (DO IGP): four NT males introduced together with the females; (iii) Cannibalism after oviposition (Post-oviposition CNB; PO CNB): four MP males introduced after females were removed; (iv) Intraguild predation after oviposition (Post-oviposition IGP; PO IGP): four NT males introduced after females were removed; and (v) Control cages were also set up with only 4 MP females and without adding NT or MP males; All NT and MP males were allowed to prey on MP eggs for four days. After the allocated time, all mirids were removed, and the plant was inspected thoroughly. No nymph was observed, which confirms that males only had access to eggs during the experiment. Each treatment was repeated 16 times.

After ten days from the beginning of the experiment, tomato plants were cut in five sections; each with one leaf and each of them was kept in 100 mm plastic glasses for five more days to allow larvae to emerge from eggs. Each cutting was provided with water in order to remain moistened, and E. kuehniella eggs were provided ad libitum in the plastic glasses to feed hatching larvae and prevent cannibalism among them. The whole experiment was done in climatic chambers (25°C, 70% RH, 16 : 8 h L : D). Under these conditions, the incubation of MP eggs lasts about 11 days (Perdikis & Lykouressis, 2004; Castañé & Zapata, 2005). Consequently, no eggs had hatched before the plant was cut, and nymphs found on each part came from eggs laid on these parts.

The number of nymphs hatched was counted five days after cutting the tomato plants and used as a proxy for the number of eggs laid by females minus those consumed by potential predators (or cannibals). Directly counting the MP eggs can lead to error because they are laid in the plant tissue and are difficult to see. We tried to stain eggs to count them in the plant rather than to infer oviposition from recording the number of nymphs (see Vanderkerkhove et al., 2006) for the method), but that method was hardly reliable on large scale (i.e. the whole plant in our case). We thus inferred
the oviposition behaviour of MP females and both intraguild predation and cannibalism based on our counts of nymphs.

Statistical analysis

The number of nymphs recorded in our control treatment (four females during four days) resulted from the fertilized eggs laid in the plant that successfully hatched. The level of intraguild predation or cannibalism was inferred by comparing “Post-oviposition IGP” and “Post-oviposition CNB” treatments with the control treatments. Intraguild predation and cannibalism by males was inferred by comparing the control treatment with the “Post-oviposition CNB” and “Post-oviposition IGP” treatments (which allowed MP females to oviposit just as in the control treatment, but then allowed males to feed on eggs for four days). The difference in counts of nymphs accounted for the predation on eggs by NT or MP males. The effect of intraguild predation and cannibalism on the recorded number of MP nymphs was analysed with a one-way ANOVA.

Our experimental design allowed us to infer the oviposition behaviour of MP females. In “during-oviposition IGP” and “during-oviposition CNB” treatments, MP females directly interact with NT or MP males, but not in the other treatments. Thus, any differences in the distribution of nymphs between “during-oviposition” and control treatments could be explained by the interaction between MP females and males of either species. A difference in the distribution of nymphs between the “Post-oviposition” and the control treatments indicated that intraguild predation or cannibalism modulate this distribution.

The female preferentially laid their eggs in the bottom leaves. Hence, if the female responded to the presence of MP or NT males, they could adjust the number of eggs laid in the upper part of the plant. Generalized linear models (GLM) for Poisson distributed data were used to test the number of

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nymphs emerging on the upper three tomato plant leaves (third leaf and the two apical leaves) in function of the treatments. The p-value of the variable was obtained using *drop1* function which runs a likelihood ratio test (LRT). Tukey’s all-pairwise comparisons of means was implemented using *glht* function (package multcomp; Hothorn et al. (2016)) to detect differences among treatments (Herberich et al., 2010; Crawley, 2013). All analysis were run in R (R Core Team, 2017).

**Results**

*Effect of intraguild predation and cannibalism on oviposition rates of MP*

The four MP females in the control tests generated an average of 22.50 (± 1.96 s.e.) nymphs in four days (Fig. 1). The post-oviposition cannibalism (PO-CNB) (14.87 ± 2.20) treatment had a significant negative effect on the number of nymphs compared to the control treatment ($F_{4, 72} = 2.64, P = 0.04$) (Fig. 1).

The number of nymphs emerged when females oviposited in the presence of four NT males (DO IGP) was 16.25 (± 1.84), and 21.27 (± 2.0) when four NT males were added after the MP females were allowed to oviposit (PO IGP).

*Effect of intraguild predation and cannibalism on egg distribution*

In the control treatment, most MP eggs were laid in the two bottom leaves of the plant (39.2% and 36.0%), whereas the three upper leaves were infrequently used (0.8%, 5.8% and 20.0% from top to bottom). The presence of potential intraguild predator during oviposition (DO IGP) had an impact on the distribution of MP eggs ($LRT_4 = 27.55; P < 0.0001$) (Fig. 2): less nymphs emerged on the upper leaves when MP females were ovipositing in presence of the NT males than in the control treatment or when NT males were added after the oviposition period.
Discussion

The eggs of zoophytophagous mirids, although inserted in plant tissues, are expectedly vulnerable to intraguild predation from other mirid species and to cannibalism. In our experiment, eggs of the zoophytophagous mirid MP were prone to cannibalism, but not significantly attacked by the intraguild predator NT. This result supports our first hypothesis that cannibalism poses a greater threat to eggs than intraguild predation. Our results corroborate those of other studies according to which intraguild predation is rare among zoophytophagous mirids (Lucas & Alomar, 2001; Lucas & Alomar, 2002; Salas Gervassio et al., 2017).

Our study was conducted under conditions of high population density and low quality food as bugs had only access to the tomato plant and mirid eggs as food resources, and this may have increased the risk of cannibalism (Hamdi et al., 2013). Hamdi et al. (2013) observed that about 60% to 70% of MP individuals tested were cannibals when prey was not available. Although zoophytophagous mirids can feed on the plant when prey are scarce (Reding et al., 2001; Castañé et al., 2011; Aubry et al., 2016, 2017), they need prey and cannibalism can act as substitute to prey. Hence, cannibalism may regulate zoophytophagous predator populations in conditions where zoophytophagous predators are more detrimental to crop than useful. Based on the similarity between the level of cannibalism in artificial and more natural conditions, Hamdi et al. (2013) suggested that cannibalism could be genetically determined. Furthermore, in the mullein bug Campylomma verbasci (Meyer) (Hemiptera: Miridae), another zoophytophagous mirid, highly zoophagous strains are more cannibals than lowly zoophagous strains independently of which food resources were available (Dumont et al., 2017b). The release of highly zoophagous strains of zoophytophagous predators may improve their use as biological control agents (Dumont et al., 2016, 2017a, 2018, 2019). Such release would likely increase cannibalism, which would be meaningful to lower the risk of crop damage once predators successfully reduced pest populations.
The female may postpone oviposition or select a safer area to lay their eggs in response to the potential risks. Consequently, the risk of intraguild predation and cannibalism should modulate both the number and distribution of eggs found on a plant. In our system, cannibals should encounter conspecific eggs more frequently than intraguild predators that use a different stratum. Therefore, ovipositing females should be more sensitive to the risk of cannibalism than to the risk of intraguild predation. However, the lack of differences in both the number and the distribution of eggs between the two cannibalism treatments (during or after oviposition) suggests that the MP females do not respond to the risk of cannibalism. Since, MP females did not respond to cannibalism risk, predation on eggs by conspecifics is expect to be a weak evolutionary force on their oviposition behaviour compared to competition by NT.

Our results reveal that the MP females preferentially laid their eggs on bottom leaves. This pattern is accentuated in presence of risk of intraguild predation (or competition) by NT males. In contrast, NT is known to preferentially occupy the apical part of the tomato plant (Moreno-Ripoll et al., 2012; Perdikis et al., 2014). Considering that females most often select oviposition sites according to the expected success of their offspring (Via, 1986; Singer et al., 1988; Thompson, 1988; Hatherly et al., 2009; Dong et al., 2013), MP nymphs can be expected to be more successful on lower leaves than on higher ones especially when facing competition by NT. It should be advantageous for MP females to respond to intraguild predation risk or competition by avoiding oviposition on upper leaves.

From a biological control point of view, the colonization of tomato plants by NT would not hamper the benefits obtained by released MP. Macrolophus pygmaeus and NT are likely complementary biological control agents against important tomato pests. Moreover, under condition of prey scarcity, cannibalism could allow MP to survive during a period of prey scarcity and maintain some level of population in tomato greenhouses. Therefore, MP could impact pest population before they reach the economical threshold.

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Disclosure

The authors declare that they have no conflicts of interest.

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**Figure 1.** Effects of intraguild predation and cannibalism risk on the mean number of MP nymphs emerged from eggs laid by four females during four days on a 16 cm tomato plant. The treatments consisted of four MP females: (1) Control: without NT or MP males; (2) Cannibalism during oviposition (DO CNB): four MP males introduced together with MP females; (3) Cannibalism after oviposition (Post-oviposition CNB; PO CNB): four MP males introduced four days after the MP females; (4) Intraguild predation during oviposition (DO IGP): four NT males introduced together with MP females; (5) Intraguild predation after oviposition (Post-oviposition IGP; PO IGP): four NT males introduced after MP females had oviposited (and had been removed). Error bars represent standard errors. Letters represent statistically different treatments.
Figure 2. The mean number of MP eggs (inferred from the number of emerged nymphs in each plant leaf) laid in the upper three leaves of young tomato plant in response to five treatments. The treatments consisted of four MP females: (1) Control: without NT or MP males; (2) Cannibalism during oviposition (DO CNB): four MP males introduced together with MP females); (3) Cannibalism after oviposition (Post-oviposition CNB; PO CNB): four MP males introduced four days after the MP females; (4) Intraguild predation during oviposition (DO IGP): four NT males introduced together with MP females; (5) Intraguild predation after oviposition (Post-oviposition IGP; PO IGP): four NT males introduced four days after MP females (and had been removed). Error bars represent standard errors. Letters represent statistically different treatments.