J Insect Behav (2015) 28:535–543
DOI 10.1007/s10905-015-9523-3

Patch Assessment for Oviposition by a Predator: the Effect of Prey Density and Prey Oviposition Period

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Revised: 22 September 2015 / Accepted: 23 September 2015 /

Abstract Density dependent numerical response is considered advantageous for biocontrol agents. Although density dependent behaviors have been examined in aphidophagous ladybirds, they haven't been studied for coccidophagous ladybirds. The relationship of prey density and prey oviposition stage on the aggregating behavior and oviposition of the coccidophagous predator *Nephus includens* (Coleoptera: Coccinellidae) was empirically investigated. In a semi-field experiment potted citrus trees were artificially infested with *Planococcus citri* (Hemiptera: Pseudococcidae) prey in order to obtain two densities, one mealybug and ten mealybugs per tree. Released *N. includens* adults were found to aggregate at citrus trees with high prey density. In a subsequent laboratory experiment four prey densities of *P. citri* were used ranging from 1 to 8 adult mealybugs at 3 ovipositional stages, a) at the young gravid female stage, b) at mid oviposition (half of egg load oviposited) and c) at the end of the ovipositional period (full egg load oviposited). *Nephus includens* females increased their oviposition rate as prey density increased and as prey oviposition progressed. It is hypothesized that this behavior of the predator might be advantageous for offspring survival and the efficacy of *N. includens* as a biological control agent against mealybugs.

Keywords Coleoptera · coccinellidae · pseudococcidae · *Nephus includens* · oviposition

Introduction

Predators that respond numerically by aggregating attacks or by ovipositing in patches with high prey density are promising biocontrol agents (Mills and Lacan 2004).

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However, the outcome of this aggregating behavior in stabilizing predator–prey dynamics has created considerable debate (Godfray and Pacala 1992; Hassell and May 1973; Murdoch and Briggs 1996; Murdoch et al. 1985). Several studies have examined aggregating responses of predators to prey density using ladybirds (Coccinellidae). In some studies positive numerical responses by coccinellid species have been reported (Elliott and Kieckhefer 2000; Evans and Youssef 1992; Kareiva 1990) while other studies on the same or other species have not shown a similar response (Coderre et al. 1987; Schellhorn and Andow 2005).

The optimal foraging theory is also applicable to ovipositing female insects that seek suitable sites to lay eggs (Mangel 1987). A foraging female seeking oviposition sites should accept a patch if it is above a certain level of quality or she might choose to leave the patch in seeking for future fitness increment (Mangel 1987). This assumes that foraging females are omniscient to their environment at some extent (Bernstein et al. 1991). Upon arriving to a specific patch ovipositing females should be capable of assessing its quality in terms of supporting survival of their offspring. This is an important decision that can greatly affect their fitness. For aphidophagous ladybirds an important aspect of patch quality that determines the survival of their offspring is prey density and the developmental stage of the prey colony.

Prey density determines the magnitude of interspecific and intraspecific competition for prey and thus the probability of offspring survival. Apart from prey density, prey colony structure has also been shown to be an important factor for ovipositing females (Hodek and Honěk 2009). Prey quality assessed as prey age and prey mixtures versus single prey instars, affects the behavior of an ovipositing female (Seagraves 2009).

For aphidophagous coccinellids oviposition has been shown to occur above a certain level of prey density (Dixon 1959, 2000). Additionally, aphidophagous coccinellids avoid egg-laying when aphid colony abundance has reached its peak (Dixon 2000; Hemptinne et al. 1992). As a consequence female coccinellids lay most of their eggs within a short period of time in the early stages of an aphid colony, which has been named the egg window (Kindlmann and Dixon 1993).

The present study addresses patch quality assessments by a coccidophagous predator based on prey colony density and age structure of its prey. Our system consists of the mealybug *Planococcus citri* Risso (Hemiptera: Pseudococcidae) and the coccinellid predator *Nephus includens* (Kirsch) (Coleoptera: Coccinellidae). *Planococcus citri* is a widespread mealybug pest species of many economically important plants (horticultural to ornamental) worldwide (Franco et al. 2004). Yield losses occur when mealybugs infest fruit or excrete honeydew that covers the fruits and leaves, often resulting in sooty mold growth, defoliation and sunburned fruit (Walton and Pringle 2005). *Nephus includens* is a predatory ladybird that preys upon *P. citri* and other mealybug species (Katsoyannos 1996). *Nephus includens* is a native widely distributed species in Greece, Italy, Spain, and Portugal (Kontodimas et al. 2007; Magro et al. 1999; Milonas et al. 2011).

In this study we (1) compared the aggregative response of *N. includens* to *P. citri* densities, (2) examined the effects of *P. citri* density on oviposition by the predator and (3) determined how prey oviposition time affected the oviposition behavior of the predator.

Materials and methods

Study organisms

Planococcus citri mealybugs were collected from *Citrus* sp. (Sapindales: Rutaceae) at Galata (Troizinia-Greece) and reared in the laboratory on potato sprouts (*Solanum tuberosum* L., Solanales: Solanaceae) at 25±1 °C, 16L: 8D photoperiod and 65±2 % RH, in large plastic boxes (30×40×15 cm) tightly covered with fine muslin.

Nephus includens was originally collected in 2007 from citrus plants in Preveza County (Northwestern Greece), infested with *P. citri.* The predator cultures were maintained in the laboratory at 25±1 °C, 16L: 8D photoperiod and 65±2 % RH, in large cylindrical Plexiglass cages (50 cm height×30 cm diam.) tightly covered with fine muslin that contained 8–12 potato sprouts infested with *P. citri* prey.

Experiment A: Aggregation behavior of *N. includens*

To test the effect of prey density on the aggregation of *N. includens* adults, a semi-field trial was designed. Potted *Citrus aurantium* L. (Sapindales: Rutaceae) trees (1–1.5 m height) were infested using ovisacs from *P. citri* mealybugs reared under laboratory conditions to create two levels of prey density. A high prey density tree was infested with 10 ovisacs and a low prey density tree with 1 ovisac. Mealybugs were allowed to establish and when young individuals reached adulthood, infested trees were used in the experiments. Six trees, 3 of each prey density level were placed in a field cage of 2×2×2 m covered with wired net. Trees were placed in two rows randomly. In each of the three cages 100 *N. includens* adults were released in the morning at 8 am. Starting 2 h later and at hour intervals afterwards till 6:00 pm the number of adults found on each plant was recorded. Additional counts before sunset and in the morning of the next day were taken. Experiments were conducted during the summer of 2009 at the Benaki Phytopathological Institute in Athens, Greece.

Experiment B: Oviposition by *N. includens* adults

Experiments were conducted on squash plants (*Cucurbita pepo* L.) (Cucurbitales: Cucurbitaceae). Seeds were sown in a half and half mixture of perlite and turf. Seeds were placed at 2 cm depth in plastic pots of 5 cm diameter and 8 cm height. Pots were kept in a greenhouse until the seedlings reached the 2-leaf stage. All plants used were at the 2-leaf stage and about 8 cm height. Experiments took place in a laboratory room with constant temperature of 25 °C and RH approximately 50 % with a long day photoperiod 16:8 (L:D). Plants were covered with a plastic cup (10 cm height, 5 cm diam.) that was sealed on top with a plastic lid with a hole of 2 cm diameter covered with fine muslin. A factorial design with two factors was used to examine *P. citri* density and oviposition status. Plants were allocated to four adult females *P. citri* densities (1, 2, 4, 8) and at each density *P. citri* females were either young gravid *P. citri* females (1) before starting oviposition (day 1), or (2) females at the 4th day of their oviposition period or (3) females at the 7th day of their oviposition period. An adult *P. citri* female in our cultures had an oviposition period of almost 1 week.

On each plant with the various combinations of mealybug densities and oviposition period, a single mated *N. includens* female was placed at the beginning of the photophase. Mated females 5 to 10 days old, were collected by observing mating pairs and collecting the females immediately after mating. Female predators were kept in plastic Petri dishes without access to mealybugs for 1 day before being used in the experiments to ensure a high level of oviposition rate. The next day the predator was removed and the eggs laid were counted. Fifteen replicates were established for each treatment combination however in order to get the final numbers for the experiment many more females were allowed to oviposit.

Statistical Analysis

A repeated measures ANOVA was used to determine the influence of prey density and time of the day on the aggregation behavior of *N. includens* adults with time as the within subject factor and density the between subject factor (Sokal and Rohlf 2012).

The influence of adult *P. citri* prey density and *P. citri* oviposition period on the ratio of ovipositing predators was tested with generalized linear model for binomial data with density and oviposition period as predictor variables. An ANOVA model was applied in order to discriminate effects of prey density and mealybug oviposition period on the total number of eggs laid by *N. includens* females and the number of eggs laid by *N. includens* females per individual mealybug. ANOVA was followed by a simple main affect analysis for each main factor to interpret the influence of density within each level of oviposition period and vice versa. Data were sqrt (x +1) transformed in order to meet ANOVA requirements for normality and homogeneity of variances. Interaction term was removed from the final model when found not significant (Sokal and Rohlf 2012).

Results

Aggregation experiment

Density significantly influenced the aggregation of *N. includens* adults ($F=6.9$; $df=1, 16$; $P=0.018$) and time of the day ($F=3.03$; $df=10, 160$; $P=0.002$) while interaction of time and density did not have a significant effect ($F=0.25$, $df=10, 160$; $P=0.99$) (Fig. 1).

Adult prey density and oviposition stage

The ratio of ovipositing *N. includens* females ranged from 0.33 to 0.87 (Fig. 2). The overall test was found highly significant ($\chi^2=15.4$; $df=5$; $P=0.009$). There was a significant effect of *P. citri* prey density on the ratio of ovipositing female *N. includens* predators ($\chi^2=12.5$; $df=3$; $P=0.006$) but not of mealybug oviposition period ($\chi^2=3.5$; $df=2$; $P=0.178$). The interaction term was also not significant ($\chi^2=10.9$; $df=6$; $P=0.09$).

There was a significant interaction effect of prey density and oviposition period of *P. citri* females ($F=3.59$, $df=6, 168$; $P=0.0022$) on the mean number of eggs that

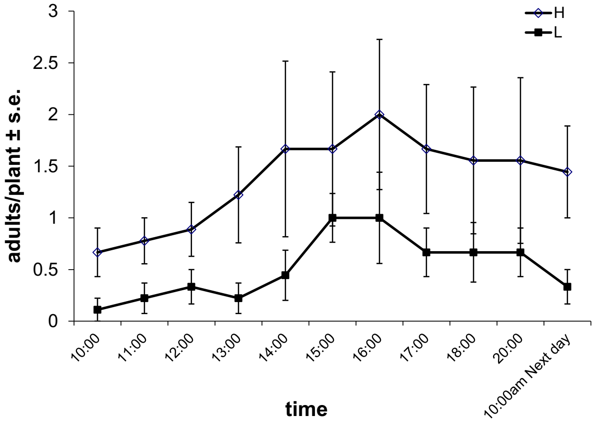

Fig. 1 *Nephus includens* adults per plant in a field cage with high and low prey density per plant (High density started with 10 *P. citri* ovisacs and low density with 1 *P. citri* ovisac)

predators laid. However, a simple main effect analysis revealed that at later prey oviposition stages (4th and 7th) the number of predator eggs increased significantly with prey density (F_{4th}=5.19; df=3, 168; *P*=0.002; (F_{7th}=13.9; df=3, 168; *P*<0.001) (F_{1st}=1.39; df=3, 168; *P*=0.248). At higher prey densities (4 and 8) the predator laid a significantly larger number of eggs on prey at more advanced oviposition stages (F_4= 4.44; df=2, 168; *P*=0.013), (F_8=6.97; df=2, 168; *P*=0.001). At the density of eight mealybugs, a higher number of predator eggs was oviposited when they were at the 4th and 7th day of their oviposition period. At the density of four mealybugs, a higher number of predator eggs was oviposited when they were at 7th day of their oviposition period (Fig. 3). No differences were detected when there were present one or two mealybugs.

The average number of eggs laid per prey is shown in Fig. 4. There was a significant interaction effect of prey density and oviposition period (F=3.14; df=6, 97; *P*=0.007) on the number of eggs laid per prey. The simple main

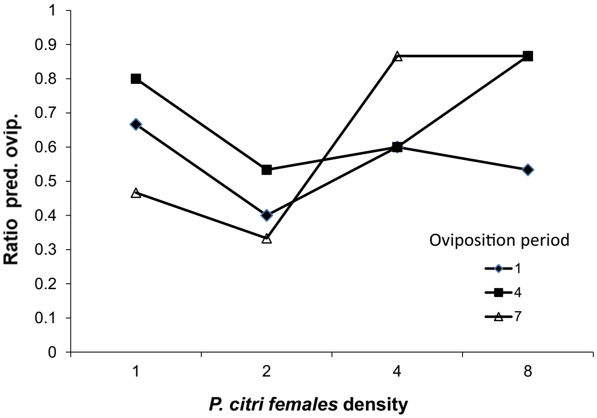

Fig. 2 Ratio of predators that oviposited during the experimental trials at different prey densities. One (1) is for young gravid *P. citri* females, four (4) for *P. citri* females at their mid oviposition stage and (7) for *P. citri* females at the end of their oviposition stage

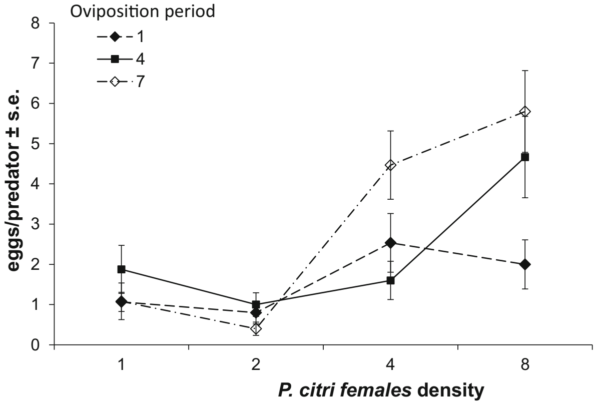

Fig. 3 Number of eggs per predator at four different prey densities One (1) is for young gravid *P. citri* females, four (4) for *P. citri* females at their mid oviposition stage and (7) for *P. citri* females at the end of their oviposition stage

effect analysis showed that the number of predator eggs per mealybug, on the density of one and four mealybugs, was affected by the oviposition period of the mealybug ($F_1=3.49$; df=2, 97; $P=0.034$) ($F_4=4.14$; df=2, 97; $P=0.019$). At the densities of 2 and 8 mealybugs, no significant influence of the mealybug's oviposition period was found ($F_2=1.45$; df=2, 97; $P=0.239$) ($F_8=2.73$; df=2, 97; $P=0.07$). In addition, simple main effect analysis showed that mealybug density had a significant influence on the number of eggs per mealybug oviposited by a *N. includens* female, for each oviposition period of the *P. citri* females ($F_1=5.6$; df=3, 97; $P=0.001$) ($F_4=18.2$; df=3, 97; $P<0.001$), ($F_7=8.2$; df=3, 97; $P<0.001$). For each oviposition period, the number of predator eggs laid per mealybug decreases with prey density. Higher number of eggs per mealybug is found at a late oviposition stage at the density of a single mealybug.

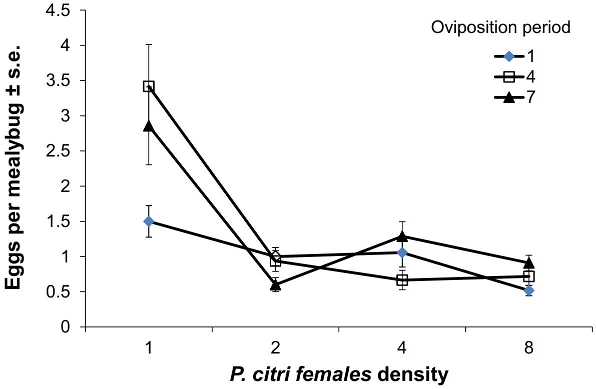

Fig. 4 Number of *N. includens* eggs per prey item at four different prey densities One (1) is for young gravid *P. citri* females, four (4) for *P. citri* females at their mid oviposition stage and (7) for *P. citri* females at the end of their oviposition stage

Discussion

Following our study, *N. includens* adults aggregate in patches with high prey density even at a small spatial scale. In general, coccinellids are known to aggregate in areas of high prey density as a result of local search patterns (Seagraves 2009). This general belief is based on numerous studies on aphidophagous coccinellids (Dixon 2000; Evans and Toler 2007; Ives et al. 1993; Schellhorn and Andow 2005). However, this is also driven by the magnitude of prey specialization of the predator. Specialists are known to thrive in lower prey densities than generalist (Sloggett and Majerus 2000). Our data show that similar aggregative behavior is likely to be exhibited also by coccidophagous predators. Aggregative responses of aphidophagous ladybirds have been shown to occur at the level of individual plants (Schellhorn and Andow 2005) and also at large-scale habitat units (Brown 2004). Here, the aggregative response was only examined at individual plant level. It is likely that *N .includens* adults respond different at larger scales like an entire field. In the field, coccids tend to form smaller colonies than aphids and more aggregated (Borges et al. 2006) and coccidophagous ladybirds like *N. includens* are expected to follow a similar pattern like their prey as life histories of ladybirds are believed to have evolved in response to those of their prey (Borges et al. 2011). In any case, aggregative responses of coccidophagous predators would have important implications for the pest management of mealybugs (Murdoch and Briggs 1996).

In the present study, *P. citri* prey density and oviposition period had an effect on the oviposition behavior of the predator. *Nephus includens* females showed increased egg laying in response to increased prey density and progress of the prey oviposition period. Although the later could be viewed as an increase of prey density as well. In any case, prey density had a positive influence on the number of eggs an individual predator laid. This density dependent numerical response is advantageous for the successful suppression of mealybug prey under biological control programmes. In combination with the aggregative behavior observed under the semi-field conditions, *N. includens* adults can be a potentially effective biological control agent against the citrus mealybug.

In aphidophagous coccinellids there is accumulating evidence that there is a density of aphids below which ladybirds are unlikely to lay eggs (Dixon 2000). Such a response had not been tested for coccidophagous coccinellids (Dixon 2000). In the current study *N. includens* females showed a density-dependent tendency in egg-laying behavior. In addition, egg-laying was affected by the oviposition period of the ovipositing female mealybugs. In aphidophagous ladybirds, females will lay their eggs when an aphid colony is rather young and the density of aphids is adequate to sustain the development of the ladybird offspring. Aphidophagous females avoid laying eggs when their egg could be consumed by conspecifics (Doumbia et al. 1998; Hemptinne et al. 1992). This is driven by the relatively rapid growth of an aphid colony (Dixon 2000). In contrast, coccid colonies are stable over time and space and provide more reliable food resources to their predators. *Nephus includens* oviposit one to two eggs per adult mealybug even when there is no prior egg-laying by the mealybugs. For a first instar *N. includens* larva it will be difficult to prey upon an adult mealybug. However, hatching for *N. includens* eggs takes as many as 5 days under constant temperature of 25 °C (Kontodimas et al. 2004). In the meantime, female mealybugs will start ovipositing and the young coccinellid larva will have enough to prey upon. Therefore, *N. includens* females exhibit strong numerical responses in relation to prey density. Moreover, at a late stage of mealybugs' ovipositional progress, predators are more likely to lay more eggs than when mealybugs are at the onset of their oviposition period.

A predator is considered as a potentially good biocontrol agent when many individuals aggregate and oviposit in areas of high prey densities. Our results showed that *N. includens* exhibit an aggregation behavior towards prey density. In addition, *N. includens* females oviposit at different stages as a mealybug colony develops and exhibit higher oviposition rates as mealybug density increases. This behavior has direct implications in biological control by coccinellids (Oliver et al. 2006). Aggregative response at high prey densities is desirable for stabilizing prey abundance at low levels. Although, other factors such as oviposition deterring pheromones by conspecific larvae may hamper the aggregative response of a coccinellid predator (Doumbia et al. 1998).

Acknowledgments This work was conducted with the financial support from the General Secretariat of Research and Technology of the Greek Ministry of Research under a bilateral cooperation for research of Greece and France (GSRT 228 e). We would like to thank two anonymous reviewers for their helpful comments that greatly improved the manuscript.

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