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DOI

[10.1111/een.12872](https://doi.org/10.1111/een.12872)

Publication date

2020

Document Version

Final published version

Published in

Ecological Entomology

License

Article 25fa Dutch Copyright Act

[Link to publication](#)

Citation for published version (APA):

Saitoh, F., Janssen, A., & Choh, Y. (2020). The use of volatile cues in recognition of kin eggs by predatory mites. *Ecological Entomology*, 45(5), 1220-1223.
<https://doi.org/10.1111/een.12872>

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SHORT COMMUNICATION

The use of volatile cues in recognition of kin eggs by predatory mites

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Abstract. 1. Several animal species are known to distinguish between their own eggs and eggs of unrelated conspecifics. However, the cues involved in this discrimination are often unknown. These cues were studied using the predatory mite *Gynaeseius liturivorus* Ehara.

2. Adult females of these predatory mites oviposit in clusters and avoid oviposition close to eggs laid by other females, resulting in reduced cannibalism between offspring. Because predatory mites are blind, it was tested whether volatiles of eggs were used as a cue for egg recognition.

3. Adult female predatory mites were offered volatile cues of their own eggs and of unrelated conspecific eggs, and females were prevented from contacting the eggs. Predatory mites oviposited closer to their own eggs than to unrelated eggs. This preference was observed even when one own and one unrelated egg were offered as a volatile source.

4. These results suggest that adult female predatory mites can determine kinship using volatiles released from the eggs.

Key words. Cannibalism, egg clusters, kin recognition, oviposition preference, predatory mites, volatiles.

Introduction

Egg clusters can be produced by females ovipositing several eggs consecutively (Itô *et al.*, 1982; Agarwala & Dixon, 1993; Stephan *et al.*, 2015) or by females returning to the same oviposition site several times to add eggs (Faraji *et al.*, 2002a; Saitoh & Choh, 2018a). For example, adult females of the predatory mite *Iphiseius degenerans* Berlese produce clusters of eggs on sweet pepper plants by moving between tufts of leaf hairs, which serve as oviposition sites, and flowers, where they feed on pollen and thrips (Faraji *et al.*, 2002b). In such cases, ovipositing females need to assess whether the eggs on the oviposition sites are from themselves or from other, conspecific females (from here on referred to as non-familial).

Volatile cues can be detected by arthropods from a distance. Hence, ovipositing females would save time and energy costs by using such cues. Although two recent studies on birds have

reported the ability to distinguish between volatiles of their own eggs and non-familial eggs (Golueke *et al.*, 2016; Leclaire *et al.*, 2017), no study has tested whether ovipositing females use volatile cues from eggs to recognise the presence of conspecific eggs on oviposition sites. Here, we studied this role of volatiles using the tiny predatory mite *Gynaeseius liturivorus*. Similar to other phytoseiids, these mites are blind (Sabelis & Dicke, 1985) and rely to a large extent on chemical cues while foraging.

Gynaeseius liturivorus lays one egg at a time and feeds between each oviposition and, hence, needs to return to the oviposition site to produce a cluster of eggs (Saitoh & Choh, 2018a). These egg clusters are often observed on leaf structures such as folds, veins, and hairs in crops such as green onion and soybean. Adult females avoid laying eggs on sites where other females have oviposited, resulting in reduced cannibalism among offspring (Saitoh & Choh, 2018b). Although eggs are involved in this oviposition preference (Saitoh & Choh, 2018b), it remains unclear whether the predatory mites use egg volatiles, chemo-tactile cues, or both to locate and select oviposition sites. Here, we investigated the role of volatiles in egg discrimination

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by this predatory mite. We assessed whether *G. liturivorus* distinguish between volatiles of their own eggs and non-familial eggs. This study contributes to understanding the function of volatiles released from eggs.

Materials and methods

Plants and arthropods

Kidney bean plants (*Phaseolus vulgaris* cv. Nagauzura) were grown in a greenhouse at $25 \pm 2^\circ\text{C}$, $60 \pm 10\%$ relative humidity (RH) and a photoperiod of 16:8 h L:D in pots (8.5 cm diameter, 7 cm height) filled with soil. For experiments, we used the primary leaves 10–12 days after sowing the seeds.

Gynaeseius liturivorus feeds on all developmental stages of thrips and are a potential biological control agent of these important pests. We therefore used Western flower thrips (*Frankliniella occidentalis* Pergande) as prey. They were obtained from the Sumika Techno Service Corporation (Takarazuka, Japan) in 2011. They were maintained on bean leaf discs (6 cm diameter) on water-saturated cotton wool in Petri dishes (9 cm diameter, 2 cm height) and supplied with ample alder pollen (*Alnus sieboldiana* Matsum.), collected on the campus of Chiba University and processed as described by Montserrat *et al.* (2006). We used first-instar thrips larvae that had emerged from leaf discs within 24 h.

Gynaeseius liturivorus, collected from green onions at Matsudo City, Japan in April 2016, were reared on kidney bean leaves with thrips larvae and were offered new leaves with prey thrice a week. Cultures of the species were maintained separately in incubators ($25 \pm 2^\circ\text{C}$, $60 \pm 10\%$ RH, 16:8 h L:D), and each individual arthropod was randomly selected from the culture for experiments. We conducted all experiments in a climate-controlled room (conditions as above).

Oviposition preference

Experimental arenas consisted of two plastic compartments that were divided with 150- μm nylon gauze and were separated before the start of the experiments (Fig. 1a). The upper compartment (36×36 mm, 18 mm high) was used to keep the volatile source, and the lower compartment (36×36 mm, 11 mm high) served to test the oviposition preference. Because adult female *G. liturivorus* prefer to oviposit on the underside of pieces of plastic rather than on leaves under laboratory conditions (Saitoh & Choh, 2018a), we used clear plastic discs (6 mm diameter) as the oviposition substrate. To obtain clusters of *G. liturivorus* eggs, an adult female predator was maintained with 20 first-instar thrips larvae on a leaf disc (20 mm diameter) with a plastic disc (as above) for 24 h. Two plastic discs with the same number of eggs (an average of 3.15 ± 0.21 eggs) laid by two adult female *G. liturivorus* were placed on the nylon gauze in the upper compartment, serving as volatile sources, with the side with eggs facing downwards. An adult female *G. liturivorus* that had laid eggs on one of the two plastic discs was used to test oviposition preference in the lower compartment. A leaf disc (20 mm diameter) on water-saturated cotton wool, serving

as a barrier against escapes, was placed in the lower compartment, and two clear plastic discs (6 mm diameter) were placed on the leaf disc as oviposition substrates, 4 mm apart. The adult female *G. liturivorus* was released in the centre of the leaf disc in the lower compartment along with 20 first-instar thrips larvae. Subsequently, the upper compartment with the volatile sources was placed on the lower one, with each of the two plastic discs with eggs in the upper compartment located immediately above one of the two clean plastic discs in the lower compartment (Fig. 1). After the system was closed, there was little airflow in the compartments. The length of an adult female is ca. 0.4 mm; eggs are approximately 0.2 mm. Hence, the distance between the two plastic discs was sufficient for the tiny mites to perceive the volatile cues from each volatile source separately, as confirmed by our results. Thus, predatory mites were offered the choice between oviposition on a disc close by their own eggs or non-familial eggs. Because of the water barrier surrounding the leaf disc in the lower compartment, the female could not reach the eggs but could only perceive volatiles emanating from the plastic discs with clusters of eggs in the upper compartment. The number of eggs oviposited by the female on each plastic disc in the lower compartment was assessed after 24 h. Egg predation by thrips larvae (Janssen *et al.*, 2002; Choh *et al.*, 2017) was not observed. There were 20 replicates (adult females), and the numbers of eggs were compared between the two plastic discs using a generalised linear model (GLM) with a Poisson error distribution.

In a second experiment, we tested whether adult females responded to cues of a single egg. The experimental arena was as above, but the volatile sources consisted of one disc with a single egg from the female tested and the other with one egg from another female. The egg was taken from other plastic discs on which females had previously oviposited for 24 h using a thin brush and was placed onto a clean plastic disc. Hence, while the discs of the previous experiment might have contained other cues produced by the predator and the thrips larvae, the discs in the current experiment only harboured an egg as volatile source. Further experimental procedures and statistics were as above.

Results and discussion

Adult female predators oviposited more on plastic discs under discs with their own eggs than on discs under discs with eggs of other females (GLM, $\chi^2 = 31.8$, d.f. = 1, $P < 0.001$, Fig. 1b). A similar preference was found when a single egg served as the volatile source (GLM, $\chi^2 = 11.2$, d.f. = 1, $P < 0.001$, Fig. 1b). Hence, these predators appear to use egg volatiles to discriminate between their own eggs and non-familial eggs. Previous studies have also reported that insects and predatory mites can distinguish between their own eggs and non-familial eggs (Faraji *et al.*, 2000; D'Ettorre *et al.*, 2004; Omkar *et al.*, 2004; Schausberger, 2005), but the cues used to discriminate in these studies may have been volatile and/or non-volatile. To the best of our knowledge, this study is the first showing the involvement of volatile cues in the recognition of non-familial conspecific eggs. It is known that the predatory mite *Phytoseiulus persimilis* Evans responds to volatiles by actively sampling the air with its first

Fig. 1. Experimental set-up for testing oviposition preference (a) and the numbers ($\pm$ SEM) of *Gynaeseius liturivorus* eggs on two plastic discs (b), one close to a source with volatiles from eggs of the female tested (black bars) and one close to a source with volatiles from eggs of another female (white bars). The top bar refers to an experiment with several eggs as volatile source and the lower bar to an experiment with one egg as volatile source. Asterisks indicate the significance of preference: *** $P < 0.001$ (generalised linear model). [Colour figure can be viewed at wileyonlinelibrary.com].

pair of legs (van Wijk *et al.*, 2006). Adult female *G. liturivorus* might respond to egg volatiles from the upper compartment in the same way. We offered oviposition sites at a short distance from the egg clusters serving as volatile sources. Further study is needed to assess the distance over which *G. liturivorus* can detect volatiles of eggs.

For our experiments, females were randomly selected from the culture, and they will therefore have differed in age and physiological state. This may have affected the preference of the females for volatiles of own or non-familiar eggs. Although the preference for own eggs was rather pronounced (Fig. 1b), differences among adult females may have caused some variation in preference. This deserves further study.

Previous studies on insects report that eggs emit volatiles that attract egg parasitoids (Leonard *et al.*, 1987; Michereff *et al.*, 2016). Here, we present the first evidence that tiny mite eggs also emit volatiles that are perceived by adult females. Obviously, such volatiles could also be used by parasitoids and predators to find and attack the eggs, so producing volatiles obviously has disadvantages (Dicke & Sabelis, 1988). Hence, the production of volatiles can therefore only be understood if they also confer some benefit. We suggest that the egg volatiles

serve to discriminate between own and non-familial eggs and avoid sites with eggs of other females. Female *G. liturivorus* avoid ovipositing on sites with eggs of other females (Saitoh & Choh, 2018b), and cannibalism between unrelated *G. liturivorus* larvae is stronger than that among sibling larvae; hence, this avoidance will result in reduced cannibalism (Saitoh & Choh, 2018b). Kin recognition of eggs with volatile cues may be crucial for species that oviposit in clusters to save energetic costs for finding oviposition sites with own eggs and reducing competition for oviposition sites.

Acknowledgements

This work was supported by the Sasakawa Scientific Research Grant from The Japan Science Society (grant number 29-512 to FS) and the Japan Society for the Promotion of Science (Basic Research C; grant number 19K06849 to YC). We thank two anonymous reviewers for valuable suggestions. FS designed and performed the experiments. YC and AJ designed the experiments and wrote the manuscript. The authors declare no competing financial interests.

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Accepted 31 March 2020

First published online 17 April 2020

Associate Editor: Shannon Murphy