EXPERIENCING THE EFFECTS IN THE HOST DISCRIMINATION BEHAVIOR OF PREDATORY MITE, PHYTOSEIULUS PERSIMILIS

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ABSTRACT
Variation in an insect’s response to a plant as a result of its previous experience is a widespread and perhaps universal phenomenon among phytophagous insects. We studied the attraction of Phytoseiulus persimilis to the volatiles from spider mite infested lima bean leaves affected by previous experience. Predators were reared from egg to adulthood on spider mite-infested lima bean leaves (Experienced predators) attracted to volatiles from spider mite infested Lima bean leaves but predators were reared from egg to adulthood on filter paper infested with lima bean or faba bean leaves spider mite didn’t prefer infested lima bean leaves to clean air. Also we demonstrate the ability of predatory mite to learn the association of a positive stimulus (egg of T. urticae as food) or negative stimulus (food absence) with volatiles from spider mite infested lima bean or faba bean leaves.

Keywords: Conditioning, Experience, HIPV, Phytoseilus Persimilis, Tetranychus Urticae

INTRODUCTION
Learning, change in behavior after experience, is a ubiquitous phenomenon in both vertebrate and invertebrate animals. Experience of predatory mites through their rearing on different host plant species modifies their subsequent foraging behavior and it can increase efficiency of predators, with several experiences, before releasing and it is important in regulating population of their hosts. Vet and Dicke (1992) suggest that learning to respond to herbivore-induced plant volatiles is expected for a specialist natural enemy of a polyphagous herbivore. Learning can convert random search to targeted search that this increases encounter rate with the host.

Drukker et al., (2000) showed the first evidence for associative learning in predatory mites and the first case for associative learning in predatory arthropods in general. They demonstrated the ability of a predatory arthropod (the plant- inhabiting mite, Phytoseiulus persimilis) to learn the association of a positive stimulus (Herbivorous prey, Tetranychus urticae) or a negative stimulus (hunger) with chemical cues (herbivore-induced plant volatiles or green leaf volatiles). Dick et al., (1990) also demonstrated the important role of previous experience on the olfactory response of P. persimilis. They showed that predators reared on bean leaves for seven days prior to olfactory experiments, preferred volatiles related to infested bean leaves rather than infested cucumber leaves. In this present study, the effect of experience before maturity and conditioning on response of P. persimilis investigated. We showed that how experience with a given prey in the early stages of life may change foraging behavior and enhance fitness-related life history traits of adult phytoseiid mites.

MATERIALS AND METHODS

Plants and Mites: Plants (Phaseolus lunatus) were grown in plastic pots on sandy soil (9cm diameter, 7 cm depth) in greenhouse conditions (25± 2°C, 60±10% RH and 16L: 8D h photoperiod). Plants were kept in another room, infested with spider mites and used for T. urticae and predator rearing. Plants with 4 same aged leaves were used for experiments.

The predatory mites, Phytoseiulus persimilis, were reared on spider mite-infested detached bean leaves in Petri dishes (9cm diameter). Each bean leaf was put upside down on a moisturized sponge. The leaf was surrounds by water- saturated cotton so that the predators could drink water without dropping into Petri dish. The fresh infested leaves were introduced to the arena five days a week and the exploited ones were removed.
The same aged (2 days old) adult *P. persimilis* females were selected as the searching predators for the olfactory experiments.

**Olfactory Response of Predator**

A Y-tube olfactometer was used for assaying the response of the searching predators for odours associated with *T. urticae* infested lima bean and faba bean leaves. Y-tube olfactometer consisted of a 14 cm long basal arm and two 10 cm long arms, each with 2 cm inner diameter.

Predators were introduced at the starting point as a steel Y-shaped wire that was fixed at the center of the Y-tube (Sabelis and Van, 1983). The lateral arms of the olfactometer through which the odours flew towards the searching predator were connected to the odour source glass chamber (3.5 cm i.d * 17.5 cm long) by means of Teflon tubing. An air pump with a flow meter (Testo 425 Hot Wire Thermo-Anemometer) was used to adjust the airflow approximately to 41 min in each arm. Air passing over the odour sources was first filtered by activated charcoal and humidified by passing it through a 1000 ml Erlenmeyer flask with 500 ml of distilled water. The apparatus was housed in laboratory conditions (25±2°C, 60±10% RH) and oriented horizontally with arms situated 30 cm below fluorescent lights. Predators that did not pass through either arm within 5 min were excluded from the statistical analysis. The odor sources were interchanged after every five experiments in order to cancel out any unforeseen asymmetries in the set-up. 20 predatory mites were tested for each treatment. The behavioral responses were statically analyzed with *X*² test to determine whether their distribution differed from 50:50 in SAS 9.1.

1. **Assessment of the Effect of Experience before Maturity on Response of Predator**

To evaluate this effect, used three predator groups in experiments: A: predators were reared from egg to adulthood on spider mite-infested lima bean leaves; B: predators were reared from egg to adulthood on filter paper infested with lima bean spider mite; C: predators were reared from egg to adulthood on filter paper infested with faba bean spider mite. Lima bean infested leaves and clean air used as odour sources.

2. **Assessment of the Effect of Associative Learning (Conditioning) on Response of Predator**

To evaluate this effect, at first, naïve predators (were reared from egg to adulthood on filter paper infested with spider mite) compared to experienced predators (they received odour from spider mite infested lima bean leaves for 24 h.). The response of predators was tested towards spider mite infested lima bean leaves versus clean air. Second, the response of naïve predators was tested towards spider mite infested lima bean leaves versus clean faba bean leaves then naïve predators transferred into petridish with wet filter paper and deprived of food for 24 h. Humidified air carrying odour from a container with spider mite infested lima bean leaves (odour combined with a negative stimulus (food absence)). Then the response of predator towards spider mite infested lima bean leaves versus clean faba bean was tested. Naïve predators transferred into petridish with wet filter paper and egg of spider mite for 24 h. Humidified air carrying odour from a container with clean faba bean leaves (odour combined with a positive stimulus (food as food)). Then the response of predator towards spider mite infested lima bean leaves versus clean faba bean was tested.

**RESULTS AND DISCUSSION**

1. **Assessment of the Effect of Experience before Maturity on Response of Predator**

A: predators were reared from egg to adulthood on spider mite-infested lima bean leaves showed a significant preference for volatile from infested lima bean leaves over clean air (*X*²= 39.2245, df= 1, p<0.0001) (figure 1). Takabayashi and Dick (1996) reported that rearing the predatory mite *P. persimilis* on spider mite infested host plants induced a preference for these host plants. Takabayashi *et al.*, (1994) showed that the response of *P. persimilis* is affected by experience. The predator’s response to infested cucumber leaves increased gradually during seven days that it was reared on this plant. It is not known whether this is the result of learning or of physiological changes. Krips *et al.*, (1999) showed that experience with spider mite-infested gerbera leaves greatly increases the response of the predators to
spider mite-induced gerbera volailes. B: predators were reared from egg to adulthood on filter paper infested with lima bean spider mite and faba bean spider mite didn’t prefer volatile from infested lima bean leaves to clean air.

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A. Predators were reared from egg to adulthood on spider mite-infested lima bean leaves. B: Predators were reared from egg to adulthood on filter paper infested with lima bean spider mite. C: Predators were reared from egg to adulthood on filter paper infested with faba bean spider mite.

2. **Assessment of the Effect of Associative Learning (Conditioning) on Response of Predator**

Naïve predators didn’t prefer volatile from infested lima bean leaves to clean air ($X^2=1.5319$, df=1, $p=0.2158$), but the change in response due to experience with volatile of mite-infested bean leaves was...
significant and experienced predators showed a significant preference for volatile from infested lima bean leaves over clean air (figure 2) ($X^2=60.48399$, df=1, $p<0.0001$). Maleknia et al., (2013) also showed that after 24 hours of experience with rose volatiles, *P. persimilis* were able to discriminate between clean and infested plants. Drukker et al., (2000) also in an experiment tested naïve predators for their response to spider-mite infested bean leaves versus clean air. Their responses were not significant in any of their replicate experiments.

In second experiment, naïve predators didn’t prefer volatile from infested lima bean leaves to clean faba bean leaves (fig.3) ($X^2=3.375$, df=1, $p>0.05$). Drukker et al., (2000) in experiment predators were raised on washed bean- reared spider mites on filter paper, and tested as adults for their response towards mite-infested bean leaves versus clean tomato leaves. In none of their replicates a preference was found, nor when the results were pooled. These results showed that predatory mites will have had no significant experience with (the whole blend of) plant volatiles after reaching adulthood on a diet of washed spider mites on filter paper (Drukker et al., 2000). They showed that naïve predator’s showed no significant preference for odour from infected bean leaves over odour from uninfected tomato leaves.

Naïve predators that received volatile from spider mite infested lima bean leaves combined with a negative stimulus, predators acquired a significant aversion to volatile from spider mite infested lima bean leaves. The change in response due to the 24h experience with HIPV in absence of food was significant (figure 3) ($X^2=28.4091$, df=1, $p<0.0001$).

In an experiment Drukker et al., (2000) also showed that naïve predators were exposed to HIPV for 16 or 24 h in absence of prey had no preference for odour from spider-mite infested bean leaves over odour from uninfested tomato leaves.

Naïve predators that received volatile from spider mite infested lima bean leaves combined with a positive stimulus, showed a preference to volatile spider mite infested lima bean leaves (figure 3) ($X^2=24.0$, df=1, $p<0.0001$). Drukker et al., (2000) also showed that when prey presence was paired to odour from spider-mite infested bean leaves, predators preferred odour from spider mite infested lima bean leaves over odour from clean tomato leaves. Bernays and Chapman (1994) demonstrated that the insects had learned an association between the odors and availability of a key nutrient they were lacking.

![Graph](image_url)

**Figure 3:** In the Y- tube olfactometer, The choice of *Phytoseiulus persimilis* between volatiles from clean faba bean leaves and volatiles from Lima bean infested leaves with *Tetranychus urticae* a.

Naïve predators that received volatile from spider mite infested faba bean leaves combined with a positive stimulus, showed a preference to volatile spider mite infested faba bean leaves (figure 3) ($X^2=21.5918$, $p<0.0001$).
df=1, p<0.0001). Drukker et al., (2000) showed that when prey presence was paired to odour from clean tomato, a preference for odour from clean tomato leaves over odour from spider-mite infested lima bean leaves was found. Naïve predators (were reared from egg to adulthood on filter paper infested with spider mite) b. Naïve predators that received odours from spider mite infested lime bean leaves combined with a negative stimulus (food absence) for 24h. c. Naïve predators that received odours from spider mite infested faba bean leaves combined with a positive stimulus (with food) for 24h. d. Naïve predators that received odours from spider mite infested lima bean leaves combined with a positive stimulus (with food) for 24h. Our results showed that naïve predators have no preference for volatile chemical until they first perceive odours in association with a rewarding experience. Bernays and Chapman (1994) showed that some insects have an ability to improve their foraging efficiency in different ways, by positive learning. At last, this is evidence for associative learning in predatory mites.

REFERENCES