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SELF- SUPER PARASITISM BEHAVIOR OF NAÏVE AND EXPERIENCED *COTESIA VESTALIS*, A BIO-CONTROL AGENT OF *Plutella Xylostella* IN BRASSICA CROPS

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ABSTRACT

Cotesia vestalis is one of the parasitoids of *Plutella xylostella*,. They do self-superparasitism to predicted as a behavior to increase the proportion of their female offspring or as a behavior resulting from their inability to recognize previously parasitized hosts. Observations were conducted to study the behavior of experienced and naive *Cotesia vestalis*. The age of *C. vestalis* females for observation was between one to three days after emerging for both experienced and naïve, but experienced *C. vestalis* were trained to lay eggs before real observation. The observation was done in a laboratory and included five behaviors, namely number of ovipositions and host encounters, period of grooming, walking and resting. There was no significant difference in the proportion experienced and naïve *C. vestalis* females that superparasitized. The number of ovipositions and host encounters, periods of walking, grooming and resting were also not significantly different between experienced and naïve *C. vestalis*. For further research, it is recommended to observe behavior of *C. vestalis* that differ in age and after longer periods of experience training. Additional behavior such as direction preferences (leaf or patches), host preference (host health and color) can additionally be important variables to study in *C.vestalis* behavior.

Keywords: Superparasitism, *C. vestalis*, naïve, experienced, behavior

INTRODUCTION

Plutella xylostella (diamondback moth) is known for causing damage to brassica crops. Larvae of this insect feed all of above ground parts of the plant which reduces yield in quality and quantity. The most common method to combat this insect is through insecticides. However, this insect becomes more resistant to chemical and biological pesticides (Li *et al.*, 2007). Therefore, integrated pest management through biological control is developing to solve this problem. Parasitoids are effective organisms as biological control agents. Introduction of parasitoids can decrease damage caused by *Plutella xylotella* (Yang, Chu, and Talekar, 1994).

The braconid *Cotesia vestalis* is a solitary parasitoid on the diamondback moth and can superparasitize hosts. Superparasitism occurs with a deposition an egg by a female parasitoid in a host already parasitized by itself or a conspecific female (Dorn and Beckage, 2007). van Dijken and Waage (1987) in Yamada and Miyamoto (1998) suggested that self superparasitism or conspecific superparasitism is a mistake of the ovipositing parasitoid that occurs due to inability of the ovipositing female to discriminate parasitized hosts from unparasitized hosts and that it is maladaptive behavior. Darrouzet et al. (2003) stated that in solitary parasitoids the occurrence of more than one egg on a host results in competition between parasitoid larvae, leading to death of all but one offspring after competition. Hence, self-superparasitism may result in a waste of time and eggs because only one offspring can be produced per host in solitary parasitoids.

However, other researchers have insisted that in some cases superparasitism may be adaptive behavior. van Alphen and Visser (1990) in Riddick (2008) stated that self-superparasitism may be beneficial if the presence of two or more eggs per host increases the overall chance that one parasitoid will survive, if the encounter rate with healthy unparasitized hosts is low. The competition between offspring may also select for stronger and superior female offspring and may increase the proportion of female offspring (Hubbard *et al*., 1999; Riddick, 2008).

There may be a relation between superparasitism behavior and the level of experience of a parasitoid. In particular, experience in host searching and handling may be relevant. Response of parasitoid to chemical and visual cues is important in host searching. This behavior can change through repeated experience. However this experience also can be forgotten and continue change little with further experience (Vet and Dicke, 1992).

Understanding of behaviour and tendency of naive or experienced parasitoids to do superparasitsm may help to identify the effective attributes used by parasitoids to exploit host resources efficiently, and thus to assess the suitability of parasitoids in biological control of insect pests. In this experiment, we study oviposition behavior in relation to self-superparasitism of naïve and experienced *Cotesia vestalis*. It is hoped that the obtained information later can provide knowledge about development interaction between a host and a parasitoid.

The objective of this experiment was to observe the superparasitism behavior of experienced and naïve *Cotesia vestalis* and to know whether there is different behavior between them in searching and handling hosts. We hypothesized that experienced *C. vestalis* will be more selective in finding and ovipositing in hosts than naïve *C. vestalis*.

MATERIALS AND METHODS

The study was conducted on 21st January to 21st February 2010 in climate room of Entomology Department of Wageningen University. There are four different rooms used for this experiment, namely room for *Plutella xylostella*, cabbage plants, *C. vestalis,* and observation. The observation room was in a climate controlled room adjusted to the natural environment of *C.vestalis* with temperature 25^{o}C, 60% of relative humidity and supported by electronic lamps for lighting.

Materials used in this study were:

- *Plutella xylostella* as the host, were maintained on cabbage plants (Brussels sprouts) in a controlled room.
- *Cotesia vestalis* females were maintained and mixed with males in a cage with sugar water and a cabbage leaf for one to three days after emergence. Therefore, they were assumed to have mated.
- cabbage leaves
- Petri dishes
- Paint (red and white)

Observation was designed in two treatments (experienced and naïve *C. vestalis*). Each treatment was replicated 20 times.

The Preparation should be done were :

- One day before experiment, *C. vestalis* were separated into experienced and naïve treatments. Every single experienced and naïve *C. vestalis* was placed individually in a Petri dish with a piece of cabbage leaf (+/- 3cm x 3 cm) and 3 drops of honey for their food. There were three groups of Petri dish preparation:
- *Experienced Petri dish***:** consisted of honey, a piece of leaf on wet tissue to avoid dehydration of leaf cabbage, one female of *C. vestalis* exposed to second to third instars of 10 *P. xylostella* for training in laying eggs in one night.
- *Naïve Petri dish***:** consisted of honey, a piece of leaf on wet tissue, and one female of *C. vestalis*.
- *Observation Petri dish*: consisted of honey, a piece of leaf on wet tissue, and four hosts (*Plutella xylostella*) in the first instars phase. These Petri dishes will be used for real observation after two hosts were removed just before the observation.
- One day after preparation of materials, in the early morning, experienced *C. vestalis* females were moved to another Petri dish without hosts for about five hours to motivate them to oviposit in the

experiment. Five hours later, around 1 p.m., the real observation was done. Two hosts in the *Observation Petri dish* were removed, and two hosts left were painted with red and white colour, to make easier in differentiate while observing.

Observations

The individual *C. vestalis* was released into the *observation Petri dish*. This dish contained damaged leaf to give them cues in recognizing their host and two painted hosts (red and white). Observations recorded included the number of ovipositions and host encounters (when *C. vestalis* approached the hosts but did not oviposit), and searching behavior such as periods of grooming, walking and resting within 15 minutes.

Data analysis

Collected data was analyzed with Z-test at 0.05 significant levels for categorizing superparasitism behavior. To analyze number of ovipositions, number of encounter, period of grooming, walking and resting non parametric analysis were used.

RESULTS AND DISCUSSION

RESULTS

From 20 *C. vestalis* in each treatment, there were 15 experienced and 13 naïve of *C. vestalis* that did superparasitism (Figure 1). The result of statistical analyses using Z-test on the number of superparasitism in experienced and naïve *C. vestalis* was not significant (p-value= 0.14, Sd= 0.14).

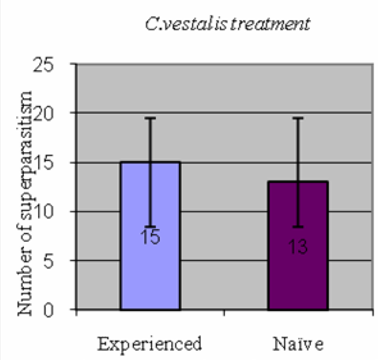

Figure 1. Number of superparasitism in experienced and naive *C.vestalis*

Table 1. Superparasitism tendency of Naïve and experienced *C.vestalis*

Category	Experienced			Naive		
	Number of *C.vestalis*	Ovipositions average	Encounter average	Number of *C. vestalis*	Ovipositions average	Encounter average
Superparasitism (S)	15	4	3.1	13	5	4.1
Non-Superparasitism (NSp)	4	1	2.5	2	1.5	2
No Ovipositions (NO)	1	0	2	5	0	2

Table 2. Periods of searching behavior (second)

Behavior	**Naive**	**Experienced**
Grooming	65.3	27.2
Resting	53.3	17.4
Walking	772.3	848.3

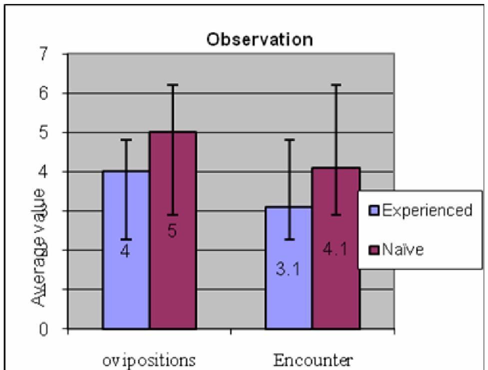

Figure 2. Average value of ovipotitions and encounter

Figure 2 shows the average number of ovipositions and encounters in naïve *C. vestalis* (oviposition= 5, encounter= 4.1) was higher than in experienced *C. vestalis* (ovipositions= 4, encounter= 3.1). Based on non parametric statistical analyses, the different number of ovipositions and encounters were not significant (p-value of oviposition = 0.15, Sd= 2 and p-value of encounter = 0.47, Sd= 2.6).

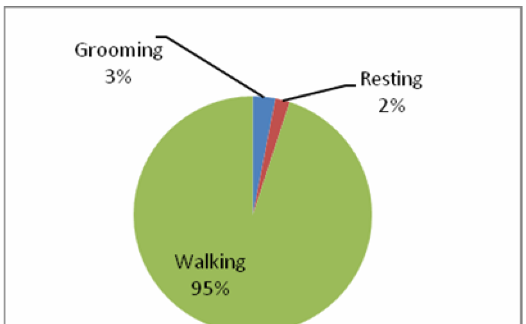

Figure 3. Experienced *C.vestalis* behavior

In Figures 3 & 4, period of grooming and resting in naïve were longer than in experienced (7% and 6% respectively for naïve and 3% and 2% for experienced), but period of walking was higher in experienced (95%). Non parametric statistical analyses showed that these differences in three variables (grooming, resting and walking) were also not significant (p-values for walking, grooming and resting were 0.05 (Sd= 113), 0.09 (Sd= 75.5) and, 0.95 (Sd= 87) respectively).

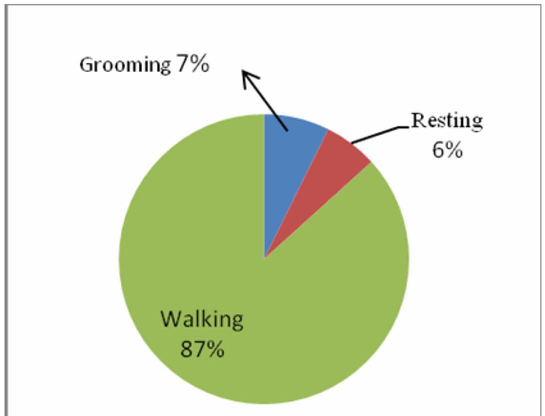

Figure 4. Naïve *C.vestalis* behavior

DISCUSSION

The result showed that both experienced and naïve of *C. vestalis* tend to do superparasitsm. The higher number of superparasitism in experienced treatment resulted from the higher number of naïve *C. vestalis* that did not oviposit at all. It might be because some naïve *C. vestalis* were not motivated to sting and probably need more time to find hosts. According to Wang *et al.* (2002), behavior of *C. plutellae* (=*C. vestalis*) was random in flying and ovipositing because they are ineffective in using volatile semiochemicals. This may also have happened in naïve *C. vestalis* in our experiment.

Before doing experiment it was expected that experienced *C. vestalis* would be more selective in parasitizing hosts after laying their eggs for about 16 hours. In contrast, naïve *C. vestalis* would be more active in superparasitizing due to their egg loads. However, the statistics result was not significantly different between naïve and experienced *C. vestalis* in superparasitism behavior. It might be because the period of training in one night was not long enough to train experienced *C. vestalis* and the number of hosts offered also too little to reduce their egg load.

The number of host encounters in experienced *C. vestalis* was higher than in naïve, but opposite was found with the number of ovipositions. It might be because experienced *C. vestalis* was familiar with the host target, which we observed with some additional behaviors. For example the experienced *C. vestalis* would directly go to damaged leave and folded leaf to search the host, but naïve *C. vestalis* were only walking around host excreta patches and doing movement similar to sting in all patches.

Naïve *C. vestalis* tends to do grooming every time they found host patches. But the experienced did less grooming. In the experiment by Wang *et al*. (2002) *C. plutella* tend to search their host in the narrow area and when they found damaged leaf they would antennate and groom. This supports our finding of naïve *C. vestalis* tending to groom every time they found the track of hosts or even host's excreta.

Naïve wasps also rested more and moved their antennae while resting; probably they tried to be familiar with the host and used their antennae to detect them. However, the period of walking in experienced *C. vestalis* was higher than in naïve *C. vestalis* although this was not significant statistically. Walking behavior in this experiment was also assumed as searching behavior. By training with 10 hosts, experienced *C. vestalis* were expected to find more hosts during the experimental period. Therefore, they kept on searching continuously by walking to search hosts.

CONCLUSION AND SUGGESTIONS

Both experienced and naïve *Cotesia vestalis* tend to do superparasitsm and there is no significant different between experienced and naïve *C. vestalis* in their superparasitsm behavior such as walking, resting, grooming, encounter and ovipositions.

For further observations, including *C. vestalis* of different ages or increasing the period of training of experienced *C. vestalis* might be helpful to get better understanding in experienced and naïve *C. vestalis* superparasitism behavior. Moreover, additional behavior such as direction preference (leaf or patches) and host preference (host health and color) could be valuable information in observing superparasitism behavior.

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