



Behavioral Responses of *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae) to Honey-Based Diets

O. J. Soyelu^{1,2*}

¹Department of Zoology and Entomology, University of Fort Hare, Alice 5700, South Africa.

²Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife 220005, Nigeria.

Author's contribution

Author OJS designed the study, performed the statistical analysis, wrote the protocol, and wrote the manuscript.

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ABSTRACT

Perception of odors and orientation of parasitoids in the direction of food sources are essential for good performance. I investigated the olfactory and behavioral responses of naïve female *Cotesia vestalis* (Haliday), a larval parasitoid of the diamondback moth, *Plutellaxylostella* (L.) (Lepidoptera: Plutellidae), to honey and honey-beebread using Y-tube olfactometer. Moistened air was blown over the food sources to carry odor in choice tests and behavior of each tested parasitoid was observed inside the olfactometer for 10 min. The diet chosen was also documented in each case. The newly emerged (i.e., <24h old) parasitoids responded to odor sources by antennating and making a series of movements. Tested females chose honey-beebread more quickly and more often than honey, which itself was chosen significantly faster and more often than moistened air (Control). The homing behavior of the parasitoids (i.e., entrance into the odor chamber) occurred more quickly with honey-beebread compared to honey, and no female wasp entered the chamber containing air alone. The results obtained in this study suggest that honey-beebread is more attractive to *C. vestalis*. This is an indication that if incorporated into the rearing program of *C. vestalis*, beebread would not have an adverse effect on the detection and movement of parasitoids towards available diet.

*Corresponding author: Email: jlekan2001@yahoo.co.uk;

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DEFINITIONS

Grooming: Female wasps rub their antennae against their forelegs and rub hindlegs against the abdomen.

Preening: Female wasps rub their wings with the hindlegs and then rub the tarsi of the legs together.

Antennation: Vigorous movement of the antennae in different directions.

1. INTRODUCTION

Cotesia vestalis (Haliday) [= *C. plutellae* (Kurdjumov)] (Hymenoptera: Braconidae) is the most common solitary larval endoparasitoid of the diamondback moth (DBM), *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) in South Africa [1], and several parts of the world [2]. The DBM is a well-known destructive insect pest of *Brassica* crops in many parts of the world [3], capable of causing total crop loss [4]. The biology of *C. vestalis* and its laboratory rearing method are well documented [5]. Female *C. vestalis* actively searches the host patch, and lays eggs in suitable host instars preferably second and third instar DBM larvae [5,6]. After hatching, the immature parasitoid starts to feed and grow within the DBM larva until the final larval instar of the parasitoid egress from a still-living fourth instar DBM larva to pupate externally, irrespective of the host instar at oviposition [6]. The parasitized host larvae are able to survive one or two more days, but they are unable to feed, and then die [7]. Adult parasitoids usually emerge from the cocoons with some mature eggs and can immediately start the next generation by actively searching and parasitizing suitable hosts within minutes of eclosion [5].

The need to mass-produce parasitoids for classical biological control, augmentative or inundative releases as a tactic to suppress pest species has necessitated the formulation of suitable supplementary diets that could be used in the rearing facilities [8]. In the wild, adult parasitoids feed primarily by foraging for plant-derived food sources such as floral and extra floral nectar, plant sap or leached phloem sugars and homopteran honeydew [9,10]. In addition, a number of parasitoid species feed on host hemolymph to obtain dietary protein for maximizing egg production [11]. These species are said to be synovigenic, emerging with fewer than the total number of eggs they might lay in their lifetime and they, therefore, continue to mature eggs for some time in their adult life [12]. Although *C. vestalis* is weakly synovigenic, its females do not need supplementary protein sources to initiate and sustain post-eclosion egg maturation [13].

Parasitic wasps can accurately locate hosts or food by using olfactory cues, visual signals, contact stimuli and perceived sound. These cues can be used either singly or in an interactive manner to enhance precision in target location [14]. Olfaction is the primary sense used by insects to detect and locate food resources [15]. Odor signals enhance food-foraging efficiency by reducing searching time and energy expended. Information about food odor is encoded by olfactory receptor cells located in several types of cuticular sensilla. Roux et al. [16] suggested that *C. vestalis* use *Sensilla coeloconica* Type I (on antennomeres 6-15) and the numerous *S. placodea* (on all antennomeres) for odor detection. The behavioral significance of odor sources can be evaluated in the laboratory using the olfactometer [17]. When formulating a supplementary food for adult parasitoids, it is imperative to ensure that emitted cues attract the target parasitoid and that the parasitoid

shows a clear preference for the diet in a *choice* situation. These expectations were borne in mind in the present study where beebread was crushed in honey to make a protein-rich adult food referred to as honey-beebread.

Honey is a sweet and flavourful product, produced by bees from nectar and/or honeydew. It is well established that aroma of bee honey is highly dependent on volatile fraction composition, which in turn depends on nectar composition and floral origin [18]. Sugars are the major components of honey and the concentration of volatile compounds in the diet is very low. Kaškonienė et al. [19] characterized the volatile profiles of 15 honey samples of different botanical origin and one beebread sample. In total 93 compounds in honey and 32 in beebread were identified. They included different classes of chemical compounds such as alcohols, ketones, aldehydes, acids, terpenes, hydrocarbons, benzene, and furan derivatives. The composition of volatile compounds of beebread was slightly different from the composition of volatiles in honey. The difference may be influenced by the unique composition of the beebread. Beebread is a mixture of honey and pollen, therefore it is likely that pollen volatile compounds may play more important role for beebread than for honey. Unlike honey which is mostly sugars, beebread is rich in essential amino acids, carbohydrates, fatty acids, vitamins, minerals, trace elements, enzymes and hormone precursors [20].

The main task of the current study is to compare olfactory and behavioral responses of *C. vestalis* to honey and honey-beebread odor cues with a view to determining the acceptability of beebread in the diet of adult wasps. It is believed that the nutrients in beebread would enhance fitness traits in *C. vestalis*. The parasitoid species is known to respond positively to honey cues and subsequently feed on the food source but the parasitoid's response to a mixture of honey and beebread has not been assessed.

2. MATERIALS AND METHODS

2.1 Insect Culture

The culture of *C. vestalis* was established from individuals that emerged from *P. xylostella* larvae and parasitoid cocoons collected from cabbage fields at Alice (32°46' S 26°50' E, altitude 540m) in the Eastern Cape Province of South Africa. The parasitoid culture was maintained on second and third instar DBM larvae inside laboratory rearing cages (53×53×53cm) at 25±1°C, 65±5% r.h., and L15:D9 photoperiod. Honey was streaked thinly on the interior top surface of the cages to serve as a source of energy for emerging diamondback moth and water was provided in glass vials (2.5×6.0cm) with a cotton wick at the mouth. Fresh cabbage seedlings grown in a compost medium were regularly supplied in the rearing cages to serve as host plant food for the larvae. Parasitoid cocoons were collected from the rearing cages and they were monitored daily for adult eclosion. Freshly emerged female parasitoids (<24h) were isolated in separate glass jars and they were provided with water as described earlier.

2.2 Odor Sources

Honey produced by *Apis mellifera* L. from Karoo wildflowers was obtained from Speelmanskop Honey, Cradock, South Africa. Beebread was collected from honeycombs that were donated by MakanaMeaderly, Grahamstown, South Africa (www.iqhilika.co.za).

One part of the cake-like beebread was crushed in three parts of honey (w/w) to give honey-beebread.

2.3 Olfactory Trials

Preference of water-satiated freshly emerged but mated *C. vestalis* females for odor cues from honey and honey-beebread was tested in a Y-tube olfactometer following the method of Rossbach et al. [21]. The glass olfactometer had the following dimensions: Main arm 29.5cm, side arms 15.5cm, inner diameter 2.3cm and angle between two side arms approximately 90°. Each side arm had a detachable bulbous component which served as container for odor sources. Prior to the trials, wasps were kept in glass receptacles around the test area for them to acclimatize to the environment. Tests were conducted in a climate-controlled room at 25±2°C and four fluorescent tubes provided a steady illumination for the test area. The fluorescent tubes had a luminous flux of 2250lm at 25°C and an emphasis at the blue and red ends of the spectrum which made it ideal for promoting photo-biological processes in plants. In two-choice bioassays, female parasitoids were exposed to different combinations of the following stimuli:

- (1) Honey: Approximately 2g in a suspended glass vial;
- (2) Honey-beebread: Approximately 2g in a suspended glass vial and
- (3) Moistened air (control): Compressed air flowing from a cylinder and passed through a water-containing jar before entering odor chambers.

Humidified air was pushed into odor chambers at 0.09-0.10m/s to carry volatiles via the side arms into main arm of the olfactometer. A naïve female (i.e. without a previous food experience) was then introduced at the base of the main arm and its behavior inside the olfactometer was observed for 10 min. Parasitoids that went beyond the intersection and remained in one of the side arms for at least 20s were recorded as having made a choice.

Females that remained in the main arm or spent less than 20s in one of the side arms were recorded as showing no response. Those that switched between the two arms without initially spending more than 20s in one of the arms were recorded as not having made a definite choice. Each wasp was tested only once in the olfactometer. To avoid bias, odor sources were switched between the left and right arms of the Y-tube after every 5 trials and a new set of glassware was used each time. After testing a given odor combination, the olfactometer was washed, rinsed with acetone and dried in an oven at 70°C for at least 24h. At least 60 parasitoids were tested for each treatment.

Behavioral activities of introduced wasps were monitored throughout the 10 min mark using a stopwatch. In the course of responding to odor cues from a diet, some wasps went as far as entering the detachable bulbous component which housed the food. This behavior is referred to as homing. Time taken by the wasps to choose an odor source and time taken to make a homing behavior were recorded.

2.4 Statistical Analysis

Data from olfactometer assays were subjected to Chi-Square analysis [22] to determine significant differences in the number of insects that chose either of the two test odors. Time taken by the wasps to choose each treatment and the time taken to make homing behavior were subjected to one-way Analysis of Variance (ANOVA), followed by Tukey's Studentized

Range Test for comparison of treatment means ($P=.05$). Non-responsive wasps were not included in the analyses. Series of movements associated with parasitoids that made a choice and those that did not make any choice were outlined separately.

3. RESULTS AND DISCUSSION

Newly emerged *C. vestalis*, without previous food experience, showed innate ability to distinguish between air and food odor and they responded by moving toward the food sources. In separate trials, *C. vestalis* chose honey cues Fig. 1A: $\chi^2=4.50$, $P=.034$ and honey-beebread cues Fig. 1B: $\chi^2=8.91$, $P=.003$ over that of moistened air. The ability to discriminate between food and non-food sources in a Y-tube olfactometer suggests that olfaction plays a key role in foraging activities of *C. vestalis*. Perception of food odor in the airstream and movement in the direction of such are crucial for optimum longevity and thus, maximal parasitism rates in *C. vestalis*. However, in *choice* experiment involving honey and honey-beebread, *C. vestalis* showed preference for cues from the latter Fig. 1C: $\chi^2=3.95$, $P=.047$. This is an indication that a constituent of beebread might have provided the extra attracting cues. Kaškonienė et al. [19] identified dimethyl sulphide, pentanenitrile, furfural, benzaldehyde, nonanal, benzylnitrile, and decanal in both beebread and honey but a higher concentration of dimethyl sulphide and furfural was recorded in beebread. Beebread also differs from honey by containing a considerably high amount of volatile compounds that are not present in honey such as acetic acid, nonane, 1-heptadecene, 1-phenylpropan-2-ol, 3-furaldehyde, 2-heptanone, 4-ethyl-4-methyl-1-hexene, 5-hydroxymethyl furfural, tridecane and 1-heptadecene. Although the effect of each volatile compound on the behavior of *C. vestalis* is beyond the scope of this study, it is more likely that one or more of these compounds made honey-beebread more preferable to the parasitoid. Another promising way of explaining the preference for honey-beebread is to consider the volatile compounds that are common in both the parasitoid host (*P. xylostella* larvae) and honey-beebread. Roux et al. [23] detected forty volatile compounds in cuticular lipids of *P. Xylostella* larvae using GC and GC-MS analyses. These compounds act as semiochemicals that mediate host-recognition and host-acceptance by the specialist parasitoid, *C. vestalis*. In comparison to an earlier study [24], four of the detected compounds (*n*-tricosanen C_{23} , *n*-pentacosanen C_{25} , *n*-heptacosanen C_{27} and *n*-nonacosanen C_{29}) were found to be common in both *P. Xylostella* larvae and beebread. *N*-nonacosane was the most dominant volatile compound in *P. Xylostella* constituting 21.4% of the total cuticular lipid extract [23]. The stimuli that lead female parasitoids to host locations and to oviposition might also play an important role in leading them to food sources that emit similar chemical signals. Dyer [25] reported that emitted tricosane and pentacosane led honey bees to nectar- and pollen-producing flowers which served as food sources.

Time taken by the parasitoid for decision making varied with the degree of preference. Generally, wasps took less time to choose the side arm containing a preferred odor and a longer time to choose a less preferred source. It took *C. vestalis* an average of 3, 3.5 and 5 min to choose the olfactometer arm containing odor cues from honey-beebread, honey and moistened air, respectively Fig. 2A. In a similar trend, female wasps took less time (approx. 2 min) to come in contact with honey-beebread inside the odor chamber (homing behavior) and they took an average of 4.5 min to get to honey. None of the females entered the odor chamber containing moistened air alone Fig. 2B. This is a clear indication that *C. vestalis* has the ability to orientate in the direction of and locate preferred odor sources more easily without wasting much energy. The type and intensity of odor may, however, be responsible for the rate at which odors are perceived, interpreted and responded to by insects.

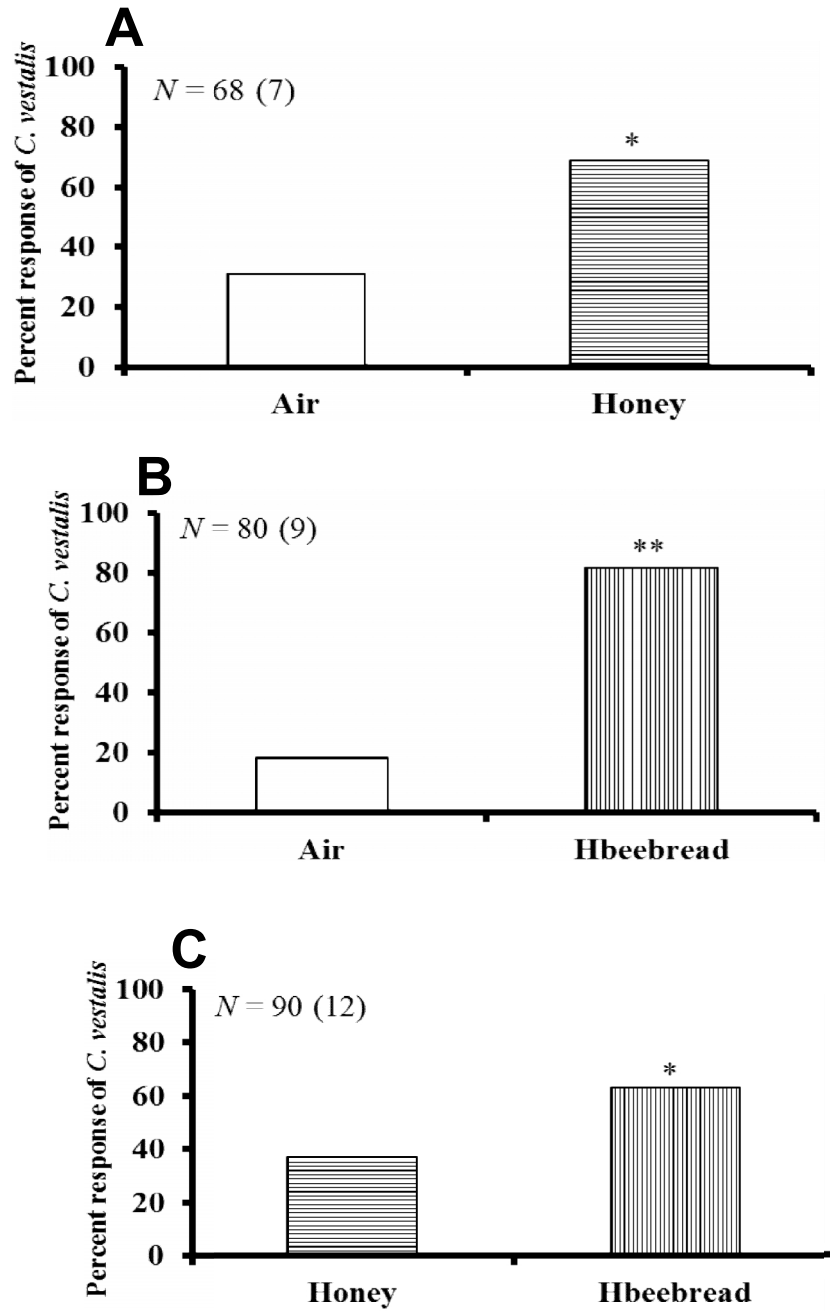


Fig. 1. Olfactory responses of naïve water-satiated *Cotesia vestalis* females toward moistened air, honey and honey-beebread. *N* = number of female wasps tested per odor combination. The number of wasps that did not respond is indicated in parentheses.

*, ** Significant χ^2 -test at 0.05 and 0.01 levels of probability, respectively.

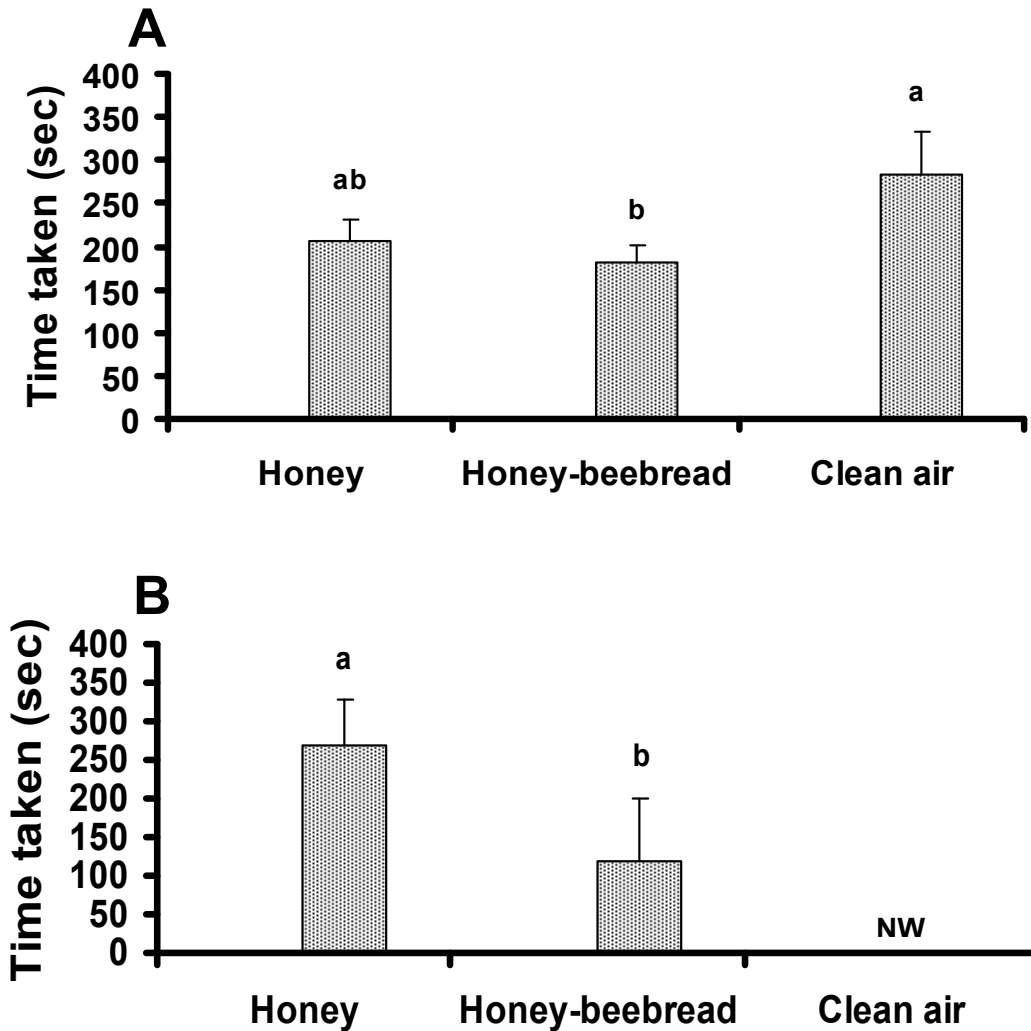


Fig. 2. (A) Average time (+standard error) spent by *Cotesia vestalis* inside the olfactometer before making a final decision for honey, honey-beebread and moistened air, respectively. (B) Time taken by *C. vestalis* inside the olfactometer before entering odor chambers (homing). No female wasp (NW) entered the odor chamber containing moistened air. Bars having different alphabets are significantly different at $P= .05$

Olfactory perception in insects is usually followed by orientation behavior which eventually leads to target location. Decision making prior to target location entails movement of different parts of the body in order for the insect to interpret perceived odor. In the present study, a complex series of movement of the antennae, wings, tarsi and other parts of the body was observed either when the insect was moving or when stationary inside the olfactometer. This is an indication that antennae alone may not be sufficient in making foraging decisions in *C. vestalis*. The pattern of movements observed in females that made a definite choice was different from those of females that did not make a choice. Generally, females that made a

definite choice spent ≤ 15 s in the receptacle (with antennae waving in an elevated position) before entering the main arm. Then, they walked rapidly along the main arm with their antennae held in U-shape position and the dorsal side of apical antennomeres (or sometimes only the tips) touching the glass. Such females also stopped once or twice for 1-3s to either groom or preen their wings. Some of the parasitoids completed their decision-making process before they got to the intersection. These ones entered the chosen arm without hesitating at the intersection and time taken from introduction into the olfactometer to positive choice was about 35s. A large number of these early-deciding wasps showed homing behavior. For other females with definite choice, there was a brief stop at the intersection with the antennae moving in all directions before they decided to enter one of the side arms. Some females hesitated midway into the side arm, antennated for a few seconds before going further into the side arm. On the contrary, few wasps left for the second arm without spending up to 20s in the arm previously chosen. Very few of the late-deciding wasps showed homing behavior and time taken from introduction to final choice ranged from 3-5 min. Female *C. vestalis* that did not make a definite choice either remained in the receptacle for more than 5 min or left after about 2 min but walked slowly along the main arm of the olfactometer. Some of these females went back to the receptacle and remained there till the expiration of 10 min. However, some wasps reached the intersection and chose to remain stationary, antennating occasionally. Such wasps either returned to the main arm or kept on moving from one side arm to the other without spending at least 20 s in any of them. Generally, female *C. vestalis* were more active and agile when exposed to odor cues from a preferred food source.

4. CONCLUSION

The laboratory assays showed that honey-beebread attracted *C. vestalis* more than honey, indicating that beebread could be incorporated into the feeding program of *C. vestalis* without the fear of masking effect. Food sources that are highly attractive are more likely to be visited more often than food sources that are less attractive. For an understanding of how honey-beebread would influence the foraging behavior of *C. vestalis* and improve its fitness in nature, it might be necessary to apply the mixture as a 'sugar spray' under semi-field conditions.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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