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INFLUENCE OF DIFFERENT ODOURS ON THE ASSOCIATIVE LEARNING OF LARVAL PARASITOID *COTESIA PLUTELLAE* (K.) (HYMENTOPTERA: BRACONIDAE)

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ABSTRACT: The ability of insect parasitoids to learn a wide range of volatiles enables them to exploit the habitats while still maintaining their searching efficiency and achieving efficient rates of oviposition. In the present study, learning of *Cotesia plutellae* adults on various inexperienced odours and their memory dynamics has been studied. The odours either repelled or acted as neutral stimulus under unexperienced condition. However, they were found to be equally efficient to associate the novel odour viz. coriander, rose, vanilla, and citral with food source in an olfactometer. After experiencing the wasps with different odours, the memory of rose and coriander odour remained for more than four hours but the memory faded on vanilla and citral within four hours. It was also found that the learning in these wasps depended on the duration of exposure to the odour while feeding on food source. The *C. plutellae* females could learn two odours at a time and responded to both odours in olfactometer when provided individually. Females did not show any preference for learned odour of vanilla and citral when both were provided in two choice condition in the olfactometer. But female preferred the rose odour over vanilla in the olfactometer after experiencing both the odour. This finding can be utilized to design the innundative release of the parasitoids. Growing coriander or rose flower in the vicinity of field and experiencing the wasps to these odours before releasing into field can arrest these parasitoids in the field which ultimately increase parasitisation and can become a key factor for the success of biological control program.

KEYWORDS: *Cotesia plutellae*, Associative learning, Rose, Coriander, Vanilla, Citral, Innate response

INTRODUCTION

Successful location of resources such as food, mate and oviposition sites by insect requires the ability to detect chemical and physical cues indicating the presence of these resources within a highly complex environment. Parasitoids have to interrupt host foraging and find food such as honeydew[6,9,13] or floral nectar[6,13,22] to obtain energy for survival, maintenance and locomotion, as well as to sustain high fertility. The provision of food resources by plants along with associated signals could play a significant role in minimizing costs of finding food by foraging parasitoids[2]. Parasitoids do not only display innate responses toward some food related signals, but they are also able to learn the most profitable cues[2] and improve the efficiency in finding food[24,25,26,30].

Dukas and Duan (2000) evaluated the conditions under which learning can have a positive effect on fitness. First, the environment must consist of patterns that can enhance performance if learned[5,23]. Secondly, insect must possess sufficiently robust sensory, learning and memory abilities that allow it to make clear associations between a stimulus and an environmental state and remember that association until the next times that association is relevant. Thirdly, learning must result in significant time savings that can be used directly or indirectly for additional reproduction.

Understanding the aspects of parasitoids foraging strategies may be crucial, especially for species that has to forage for hosts and food in different habitats. Such species will face extra costs of energy and time as they commute between host and food sites to fulfill both their reproductive and nutritional needs. Therefore, in these species, their ability to detect food sources from a distance through olfactory or visual learning would minimize time spent searching for food and will be highly beneficial[31].

Adult of *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae), a larval parasitoid of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), requires food such as nectar or extra floral nectarines to enhance their survival and fecundity. Thus**,** learning can play an important role in foraging for food[21]. So, the aim of the study was to first examine that if feeding on sucrose solution in association with different odour affects subsequent odour response in the wasps. Also, food associated learning on different odours of rose, coriander, citral and vanilla in response to time elapsed after experience, memory interference by multiple odour and at various combinations of two odours was evaluated. It was also determined whether learning in *C. plutellae* is influenced by duration of exposure to odour or first encounter with the odour associated with food.

MATERIALS AND METHODS

Insect Culture

Larvae of diamondback moth (DBM), *P. xylostella*, parasitized by *C. plutellae* were collected from cauliflower plants near Delhi (India). These were reared on cauliflower leaves in the laboratory at 25 ± 1°C under 65 ± 5% R.H.and 14L: 10D photoperiod. The pupae of the parasitoid emerging from the DBM were placed in separate jars for emergence. The adult parasitoid that emerged from pupae were transferred to a clear acrylic ventilated chamber (20 x 20 x 20 cm) and given 50% honey in water as food. These wasps were allowed to mate, and oviposit on late second instar DBM larvae which were transferred to clean jars and reared on cauliflower leaves in the absence of honey.

For each test, required number of parasitoid pupae were drawn from the culture and kept one each in a glass vial (60 mm long, 15 mm dia). One day old virgin, water satiated and food-deprived wasps were used for various tests. All tests were carried out in an orientation room with exhaust facility and maintained at 25±1°C and 65±5% R.H.

Experimental set-up Training chamber:

Training of the wasps was done in a rectangular glass chamber (75 mm long, 27 mm wide and 25 mm high). One of the vertical small sidewalls (27mm wide) was kept open for the entry of parasitoid. The top walls was covered with a glass strip (45 mm long, 27 mm wide) and bottom of the chamber was covered with nylon net (12 mesh/cm). Food swab was placed in the chamber on the bottom of netted strip. The odour was presented to wasps in the Bakelite cups (12 mm id, 10 mm depth), placed below the food source under bottom netted strip

Olfactometer to check the learning response:

Learned responses were tested in a T-shaped glass tunnel, consisted of a 75 mm long, vertical arm (10 mm bore) opening perpendicularly into the middle of a 150 mm long horizontal arm (15 mm bore), demarcating it into two side arms. Each side arm was graduated in millimeters starting from the entry arm junction. Each side arm was provided with a snugly fitting plunger which consisted of a glass vial (60 mm long, ~15 mm outer dia.) with a narrower mouth (10 mm inner dia.) at its inner end facing the entry arm junction, and a flat bottom at its outer end, attached to a glass rod (60 mm long, ~ 15 mm dia) with parafilm. The glass rod partly projected out of the side arm through its open end and served as a handle to slide the vial in and out. The T-tunnel was supported on two cylindrical stands (25 cm ht x 4 cm dia), one under each side arm, with the entry arm projecting vertically downward between these stands. The tunnel was illuminated from above by two 20 W fluorescent tube lights (60 cm each) at a height of 50 cm from the horizontal arm. The light intensity at the level of the tunnel was 450 lux.

Innate response

The freshly emerged females and males of *C. plutellae* were separated and kept in glass vial with water soaked cotton for 24 hrs. The response of these food deprived wasps was checked on the novel odour in glass T- tunnel.

Associative learning:

Test to determine learning ability

The experiment was designed to determine that insect can learn to associate food source along with novel odour. Freshly emerged males and females of *C. plutellae* were transferred individually in the glass vial (2.5 cm dia. and 6 cm ht) for 24 h and provided water, soaked in cotton swab. These food deprived wasps were used for the experiment. Adults were exposed to sucrose (20%) solution soaked in cotton swab in a feeding cage described earlier and allowed to feed. At the same time, odour source was provided below the food swab so that the *C. plutellae* can perceive the odour during feeding. The wasp was allowed to walk directly from vial to sucrose solution and allowed to feed for 5 sec, after that female was put back in the vial. The training procedure for each odour was repeated 3 times at an interval of 60 sec. Each test was done on 50 insects which were arranged in 5 replicates of 10 insects each. The odours used in the experiment were citral, vanilla, rose, and coriander. During the experiment, care was taken that females never contacted the odour sources with their antennae or other parts of their body.After 10 min, the response of individual wasp towards odour source was checked in T-tunnel olfactometer.

Length of memory

The food deprived female *C. plutellae* wasps were trained thrice on the four novel odour sources individually in the chamber during feeding of sucrose solution. Post training response of the wasps was evaluated after an interval of 10 min, 1 hr, 2hr, and 4hr in the T-tunnel olfactometer for each odour separately.

Effect of multiple experiences

The experiment was designed to determine if experience of two different odours causes interference between memories of those odours. The female wasps were trained to associate rose odour to sucrose solution (20%) for thrice and allowed to return to their vial. Thirty minutes after first experience, the wasps were again trained on the vanilla odour in the same manner as in rose. Response towards individual odour as well as odour preference between vanilla and rose was checked 30 minutes after the training procedure was completed.

Effect of first encounter and/or feeding duration

The experiment was done to find out whether the female wasp can memorize the odour during first encounter or duration of feeding is important. The female wasps were allowed to feed on sucrose solution for 30 sec. In the first five seconds, the wasp was allowed to perceive vanilla odour provided from below the food source. After that, the odour was replaced by citral without disturbing the feeding of the parasitoid. Out of 30 sec, the time of feeding duration was shifted from 5 sec to 15 sec and 25 sec in vanilla odour. The rest of time insect perceived the other odour source. Thirty minutes after the training, the response of wasps toward vanilla and citral was checked in the T- tunnel. Similarly, the experiment was repeated on rose and vanilla odour.

Effect of odours mixture

The female wasp was experienced three times to feed on sucrose solution in the presence of rose odour. Thirty minutes after experience, the response of wasp towards experienced odour was checked in the various ratios with inexperienced odour of vanilla. The ratio of experienced odour with inexperienced odour was 1:1, 1:9 and 9:1. In the same way, citral odour was experienced and response was checked with the mixture of vanilla odour in T- tunnel.

STATISTICAL ANALYSIS

In each set of experiments, the choice made by parasitoids was analyzed by student's paired t-test to find out the learning ability in the wasps. One way ANOVA was also performed which was followed by Tukey's test to find out the significance in odour preference by female wasps at different time interval after experiencing the odour as well as for various time duration of feeding. All the statistical analyses were carried out using the computer program SigmaStat 2.0 (Jandel, 1995).

RESULTS AND DISCUSSION

Innate response

The naïve *C. plutellae* adults did not positively respond to any odour provided in the T-tunnel. The female wasp was repelled by the rose, vanilla and citral while coriander acted as neutral odour (t value 15.9, -0.54, -7.48 and -9.49 respectively) (Table 1). Similarly, male wasps were also repelled by vanilla and citral while rose and coriander acted as a neutral odour (t value -2.14, 0, -3.16 and - 3/16 respectively) (Table 1).

Table 1: Innate response of male and female *C. plutellae* towards different odours.

Sex of the test wasp	Different odours in vials		Response of the wasps to vial A & vial B		t-value
	Vial A	Vial B	Vial A	Vial B	
Male	Rose	Control	42 ± 3.74	58 ± 3.74	-2.14^{ns}
	Coriander	Control	50 ± 4.47	50 ± 4.47	0.0^{ns}
	Vanilla	Control	40 ± 3.16	60 ± 3.16	-3.16^{**}
	Citral	Control	40 ± 3.16	60 ± 3.16	-3.16^{**}
Female	Rose	Control	28 ± 3.74	72 ± 3.74	-5.90^{***}
	Coriander	Control	48 ± 3.74	52 ± 3.74	-0.54^{ns}
	Vanilla	Control	22 ± 3.74	78 ± 3.74	-7.48^{***}
	Citral	Control	20 ± 3.74	80 ± 3.74	-9.49^{***}

The ratio of male and female adult emergence at *p<0.05, ** p<0.01, ***p<0.001 and ns showed no significance (P = 0.05) (Student's t-test).

Associative learning:

Test to determine learning ability

C. plutellae wasps that were trained to associate an odour with the food source got strongly influenced by the learned odour. Females significantly responded to learned odour of rose, coriander, vanilla and citral individually in the T-tunnel olfactometer, 10 minutes after the experience, otherwise acted as either a repellent or neutral odour to the wasps (Figure 1A). The ability to learn the novel odour was also observed in the male *C. plutellae* who also preferred the learned odour of rose, coriander, vanilla and citral individually in the T-tunnel (Figure 2A).

Length of memory

When the female wasps experienced the rose odour while feeding on sucrose solution in the training chamber, 1h before the test, preferred the rose odour when given the choice of rose and vanilla in T- tunnel olfactometer. The same response was observed when the test was repeated with the odour of coriander, vanilla and citral over the novel or inexperienced odour (Figure 1B). The male wasps were also found to respond in the similar manner as that of females after experiencing the odour. The male wasps preferred the learned odour of rose over inexperienced vanilla odour and also preferred experienced vanilla over inexperienced odour of citral (Figure 2B).

The length of the memory differed in the different odour tested. The different odours tested in the experiment were retained by both the sexes of wasps at least upto 2 hr of experience (Figure 1C and 2C). However, the learned odour of rose and coriander was retained upto 4 hr in the memory of the *C. plutellae* wasps. Whereas, the experience of odours of vanilla and citral was not retained by the wasp till 4 hr duration (Figure 1D and 2D). The preference index showed that the learned odour of rose was equally preferred at 1hr, 2 hr and 4hr by both male and female wasps. However, the preference index of vanilla decreased as the time interval increased from 1hr to 4 hr for both male and female wasps.

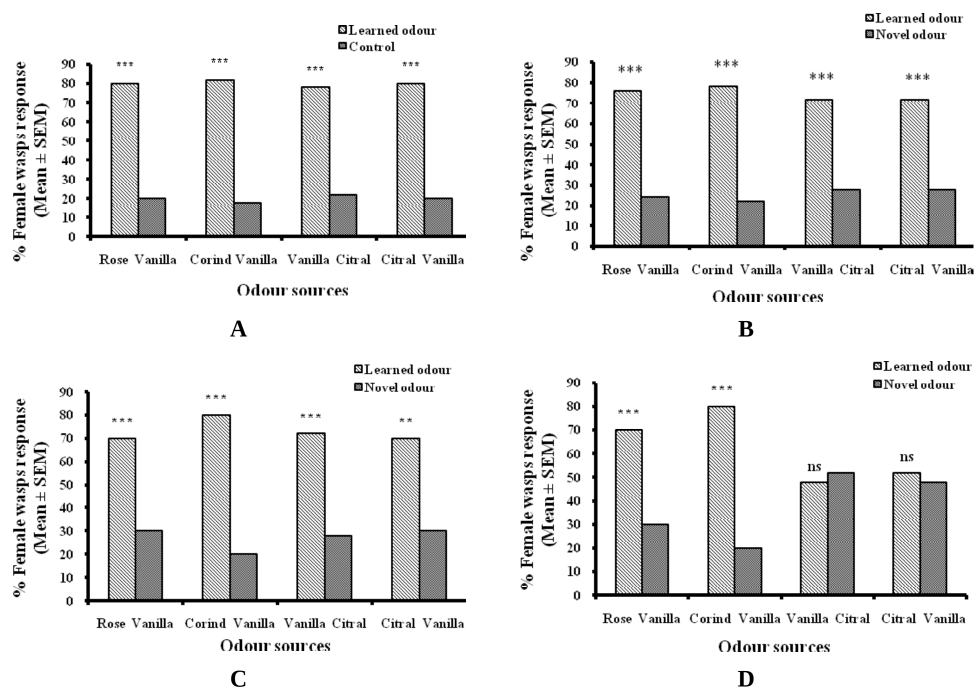


Figure 1: Learning of Female *C. plutellae* adults for different odours, tested after A)10 minutes interval, B) 1h interval, C) 2h interval and D) 4h interval. (t-value as calculated by Student's t-test for paired samples. **, *** Response significant at $p<0.01$ and $p<0.001$respectively; [ns] Difference not significant statistically)

Moreover, there was no significant difference between the sexes at the given time intervals (Figure 3).

Effect of multiple experiences

The female was allowed to experience two odour of rose and vanilla simultaneously associated with feeding and it was found that the female was able to learn and strongly responded to rose and vanilla when presented in single choice condition (t-value 9.49 and 9.49 respectively) (Table 2). However, female preferred the rose odour when presented in two choice conditions of rose and vanilla in T- tunnel olfactometer (t-value 4.81) (Table 2). The same treatment was done with vanilla and citral odour and it was found that the female was also able to learn the odour of citral and vanilla when presented in single choice (t-value 8.55 and 9.49 respectively) (Table 2). However, female did not show any preference between citral and vanilla when presented in two choice of vanilla and citral in T-tunnel olfactometer (t-value 0.54) (Table 2).

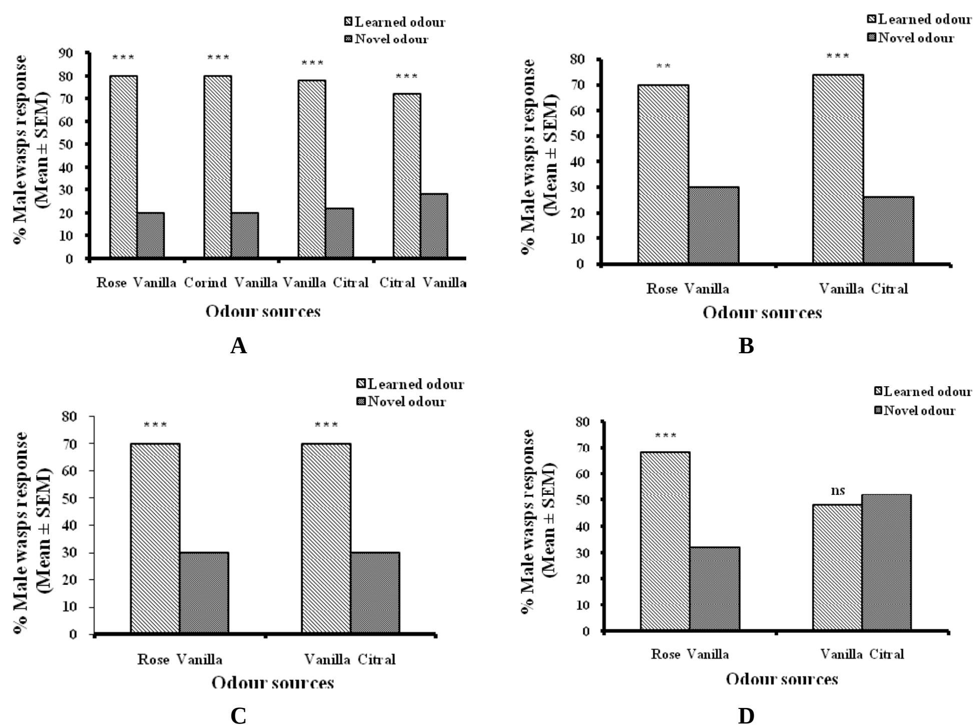


Figure 2: Learning of Male *C. plutellae* adults for different odours, tested after A)10 minutes interval, B) 1h interval, C) 2h interval and **D) 4h interval. (**t-value as calculated by Student's t-test for paired samples. **, *** Response significant at p<0.01and p<0.001respectively; ns Difference not significant statistically)

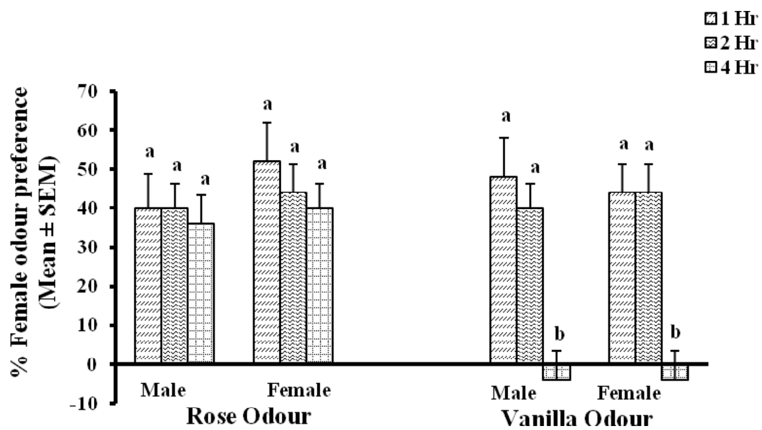


Figure 3: Preference index of female *C. plutellae* adults on vanilla odour at various duration of feeding time. (Bars with different lower case letters differ significantly (p<0.05) at learned odour).

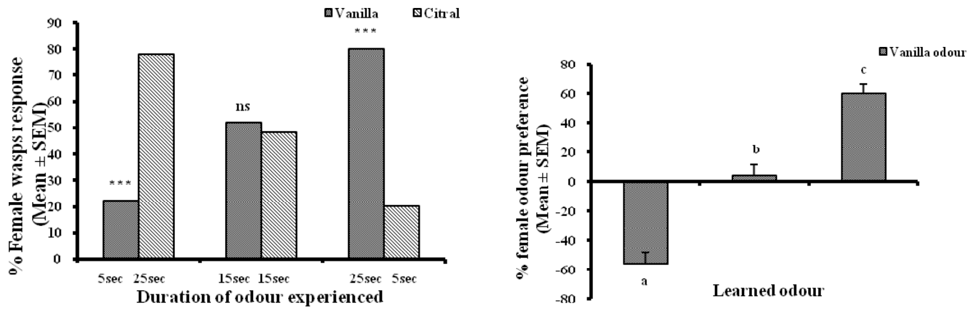
Table 2. Response of female *C. plutellae* towards two different odours experienced simultaneously during feeding.

Odour learned	Source of Stimuli		Percent response of wasps		t-value
	Vial A	Vial B	Vial A	Vial B	
Rose followed by Vanilla odour	Rose	Control	80 ± 3.16	20 ± 3.16	9.49***
	Vanilla	Control	80 ± 3.16	20 ± 3.16	9.49***
	Rose	Vanilla	68 ± 3.74	32 ± 3.74	4.81***
Citral followed by Vanilla odour	Citral	Control	82 ± 3.74	18 ± 3.74	8.55***
	Vanilla	Control	80 ± 3.16	20 ± 3.16	9.49***
	Citral	Vanilla	52 ± 3.74	48 ± 3.74	0.54^{ns}

The ratio of male and female adult emergence at *p<0.05, ** p<0.01, ***p<0.001 and ns, showed no significance (*P* = 0.05) (Student's t-test).

Effect of first encounter and/or feeding duration

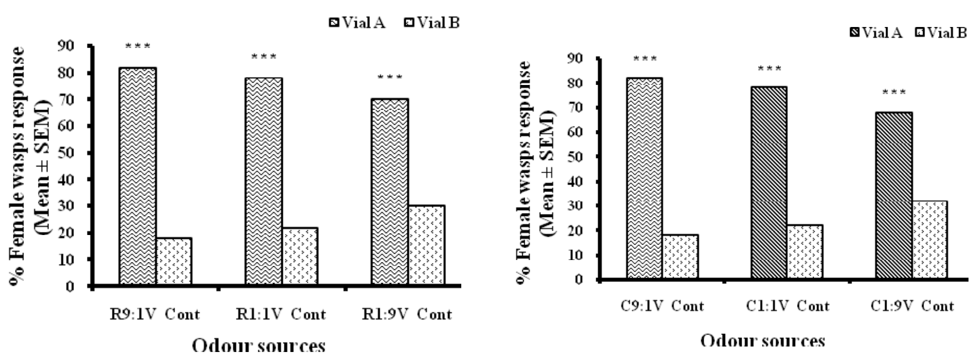
Female was allowed to feed on sucrose swab associated with the odour of vanilla and citral simultaneously for various duration of time. It was found that the odour which was perceived during feeding for longer duration was memorized by the wasp and responded significantly more toward that odour instead of first encountered odour or the odour which was perceived for shorter duration (Figure 4).



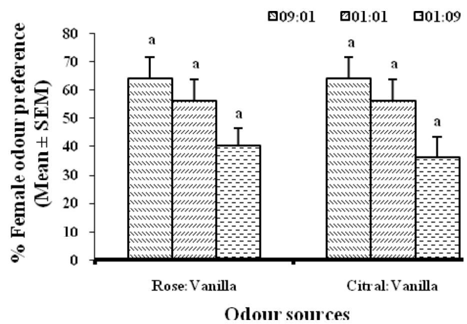
A B

Figure 4: Response of female *C. plutellae* adult wasps towards A) two different odours experienced simultaneously for various duration in total 30 seconds B) Preference index on vanilla odour at various duration of feeding time. (t-value as calculated by Student's t-test for paired samples *** Response significant at p<0.001; [ns] Difference not significant statistically. One way ANOVA was performed followed by Tukey's test. Means followed by different small letter in the column differ significantly (p<0.05).

When the female wasp was allowed to feed on sucrose cotton swab for the total duration of 30 seconds, associated first with vanilla for 5 sec and then citral for 25 sec, the female responded feebly towards the vanilla odour over citral (t-value -7.48). As the duration of feeding associated with vanilla odour was increased to 15 seconds, the response was also increased (t-value 0.54). However, the response between these two odours did not differ significantly (t-value 0.54, p= 0.05). The response reached to maximum when feeding associated with vanilla was increased to 25 sec over citral 5 sec (t-value 9.99, p<0.001) (Figure 4A). The preference index of vanilla also increased as the duration of experience on vanilla odour associated with feeding increased from 5 sec to 25 sec (Figure 4B).



A B



C

Figure 5: Response of female *C. plutellae* adult wasps towards learned odour mixed in various combinations with inexperienced odour A) learned odour of rose mixed in various combination with vanilla B) learned odour of citral mixed in various combination with vanilla C) preference index towards learned odour of rose and citral mixed in various combination with vanilla. (t-value as calculated by Student's t-test for paired samples. *** Response significant atp<0.001. One way ANOVA was performed followed by Tukey's test. Means followed by same small letterdid not differ significantly (p>0.05).

Effect of odours mixture

When rose odour was associated with sucrose swab and then response of rose odour was checked by mixing the odour with various ratio of inexperienced odour of vanilla in the T-tunnel and it was found that the female wasp was able to detect the rose odour in the mixture of vanilla even at minimum concentration. The response of female wasps was significantly higher to rose odour, when mixed with vanilla at various ratio (9:1, 1:1, 1:9 respectively) (t-value 8.55, 7.48, 6.32 respectively $p<0.001$) (Figure 5A). Similar results were obtained when above test was repeated with the learned citral odour mixed with vanilla odour in 9:1, 1:1 and 1:9 ratio (t-value 8.55, t-value 7.48 and t-value 4.81 respectively, $p<0.001$) (Figure 5B). The preference index showed that there was no significant difference within the ratio of rose odour (9:1, 1:1 and 1:9) tested in the T-tunnel as well as for citral odour ($p=0.05$) (Figure 5C).

To maintain a high survival, parasitisation and flight activity, parasitoids need continuous supply of food source in the complex environment. For this purpose, learning would be an adaptive mechanism of parasitoid to quickly respond to new profitable habitat[2,28,29]. Parasitoid wasps that had experienced an odour while feeding on sucrose solution, however, showed a strong preference for the odour which they had experienced in conjunction with the food. This odour preference after experience fits the paradigm of associative learning[9,17,20]. Sucrose solution which was innately recognized is an unconditional stimulus. The odours of rose, coriander, vanilla, and citral which were used in the present study did not elicit food searching behaviour of naïve wasps and thus these odours were conditional stimuli. The adults were able to associate the odour of all the volatiles given separately in the vicinity of food and showed the orientational response towards the odour in T- tunnel.

Not only females but males also efficiently associate the odour with food as that of females[27] because males also need food to sustain life and to searching for mates[19]. Males and females of pupal parasitoid *Pimpla alboannulatus* learned the novel odour while feeding and showed the preference for the learned odour. Olfactory learning in male is also seen in some primitive halictine bees where males learn female odour during mating and thereafter avoid them[20]. When the larval parasitoid *M. crocipes* is allowed to feed on sucrose water in the presence of the odour, they learned to associate the odour with sucrose[14,26]. The female *Pimpla luctuosa* can also learn to associate novel odour of vanilla and strawberry with its host[8].

Memory of food associated odour varied among different insect. They retain the odour which they face more frequently during feeding while others are not sustained for longer duration[12]. Bees, that had been given food associated with colour for thrice, preferred the experience colour for thirteen days[15]. In case of *C. plutellae* both male and female were able to sustain the memory of vanilla and citral odour for less than 4 hr. However, the odour of rose and coriander could be retained for more than 4 hours. The exact reason for long term memory on the rose and coriander is not known but it can be assumed that the linalool, a common floral scent[10], found in most of the flowers and also present in rose and coriander, are responsible for the innate attraction of the parasitoid. Linalool had previously been reported as attractant for honeybees[7] as well as for the parasitoid *Aphidius ervi*[3]. But the behavioral response to odour compounds is generally a function of odour concentration in which attractiveness is restricted to a certain concentration range. In the present study 0.1% concentration of coriander and rose was used which is very high as compared to the floral concentration. Due to this high concentration these odours act as repellant or neutral to wasps in olfactometer but when experienced along with food source they memorizes and respond to the odour for longer duration. In addition to concentration, initial orientation by general flower cues would help parasitoid in encountering their first nectar source. Parasitoid could subsequently incorporate responses to the specific flower odour of rewarding flower through the process of food associated odour learning[18,33]. *Cotesia glomerata* was found to visit on the flowers of *Viciasepiium, Trifolium pretense, Daucus carota* that were not attractive or even repellent in the olfactometer[32]. In case of citral and vanilla, they act as novel odour to which parasitoid does not encounter in its life. So, the association of these odours with food source makes the parasitoid to learn the odour but the learning remains for the short duration.

It has also been reported that the performance of learning is dependent on the age of parasitoid and number of times it encounters the food[17,26]. In the present study, only 24 hr old parasitoids were used for the learning experiment. In *M. crocipes*, learning ability was increased for longer duration as the age of the parasitoid increase as well as the number of experience of odour associated with food was increased[26]. The female of larval parasitoid, *Leptopilina boulardi*, showed a short term memory (1-2 hr) when single oviposition was associated with odour of banana and retain long term memory (24hr) when oviposition was associated with multiple experiences. The female *Nasonia vitripennis* showed increase in preference on rewarded colour over unrewarded with increase in number of training days[16].

The females are able to learn even more than one odour at a given time. The *C. plutellae* when learned on rose followed by vanilla, showed positive response towards rose and vanilla when given separately in the T-tunnel. However, female preferred rose odour when given two choices between rose and vanilla. Moreover, when female learned on citral followed by vanilla, also showed the same response when provided individually in a T-tunnel. But the females did not show any preference when citral and vanilla was given in two choice condition. Females of *M. crociepes* could also learn two different odours associated with food and could remember both the odour without showing a preference for one odour over the other[26]. In case of larval parasitoid *L. boulardi*, female can learn at least two different odours associated with oviposition experience and preferred the last odour learnt[1].

C. plutellae are also able to detect the experienced odour in the mixture of two or more odours. The female recognises the learned odour of rose and citral when mixed with vanilla in each case even in smaller concentration. The parasitoids have to search the food or host in the environment of complex odour[11]. Parasitoid has to detect the specific odour from the complex odour mixture for host or food searching[12].

The duration of experience, rather than first encounter of food associated with odour, plays very important role in the food learning process of parasitoid. The preference of female *C. plutellae* for the vanilla odour increases over the citral as the duration of experience for the vanilla increases in association with food. In contrast, honey bee prefers the first encountered colour associated with food rather than duration of colour[15]. The *Aphidius ervi*, when conditioned with yeast-fermented nectar, they showed attraction to the odour and memorize it up to 24 h[21].

The present study suggests that parasitoids have the ability to associate the odour with food resource. The experienced odour can be memorized for longer duration and it can be manipulated to control insect pests. The parasitoid must be experienced with the odour before innundative release. The experienced odour would then attract and bring the parasitoids to the proximity of the food which ultimately increases the parasitisation in the pest infested field; otherwise parasitoid has to devote its time for searching the food for longer distances.

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REFERENCES

1. de Jong, R. & L. Kaiser, 1992. Odour preference of a parasitic wasp depends on order of learning. *Experientia*, 48: 902-904.
2. Dridi, S. & L. Lehmann, 2016. Environmental complexity favors the evolution of learning. *Behav. Ecol.,* 27: 842–850.
3. Du, Y.J., G.M. Poppy, W. Powel, J.A. Pickett, L.J. Wadhams & C.M. Woodcock, 1998. Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi. J. Chem. Ecol.,* 24: 1355-1368.
4. Dukas, R. & J.J. Duan, 2000. Potential fitness consequences of associative learning in a parasitoid wasp. *Behav. Ecol.,* 11: 536-543.
5. Dukas, R., 1998. Evolutionary ecology of learning. In: Cognitive ecology (ed. by R Dukas) University of Chicago Press, Chicago, pp. 129-174.
6. Elliot, N.C., G.A. Simmons & F.J. Sapio, 1987. Honeydew and wildflowers as food for the parsites *Glypta fumiferanae* (Hymenoptera: Ichneumonidae) and *Apanteles fumiferanae* (Hymenoptera: Braconidae). *J. Kansas Entomol. Soc.,* 60: 25-29.
7. Henning, J.A., Y.S. Peng, M.A. Montague & L.R. Teuber, 1992. Honey bee (Hymenoptera: Apidae) behavioral response to primary alfalfa (Rosales: Fabaceae) floral volatiles. *J. Eco. Entomol.,* 85: 233–239.
8. Iizuka, T. & K. Takasu, 1998. Olfactory associative learning of the pupal parasitoid *Pimpla luctuosa* Smith (Hymenoptera: Ichneumonidae). *J. Insect Behav.,* 11: 743-760.
9. Jacob, H.S. & E.W. Evans, 2000. Influence of experience on the response of *Bathyplectes curculionis* (Hymenoptera: Ichneumonidae), a non aphidophagous parasitoid, to aphid odour. *Biol. Cont.,* 19: 237-244.
10. Knudsen, J.T., L. Tollsten & G. Bergstrom, 1993. Floral scents: a check list of volatile compounds isolated by headspace techniques. *Phytochem.,* 33:253–280.
11. Kruidhof, H.M., A.L. Roberts, P. Magdaraog, D. Munoz, R. Gols & L.E.M. Vet, 2015. Habitat complexity reduces parasitoid foraging efficiency, but does not prevent orientation towards learned host plant odours. *Oecologia,* 179: 353–361.
12. Kruidhof, H.M., F.G. Pashalidou, N.E. Fatouros, I.A. Figueroa, L.E.M. Vet & H.M. Smid, 2012. Reward value determines memory consolidation in parasitic wasps. *PLoS One,* 7: e39615.
13. Leius, K., 1967. Food sources and preferences of adults of a parasite *Scambius buolianae* (Hymenoptera: Ichneumonidae) and their consequences. *Canadian Entomol.,* 99: 865-871.
14. Lewis, W.J. & K. Takasu, 1990. Use of learned odours by a parasitic wasp in accordance with host and food needs. *Nature*, 348: 635-636.
15. Menzel, R., 1985. Colour pathways and colour vision in the honey bee (ed. By D Ottoson and S Zeki), *Central and Peripheral Mechanisms of Colour Vision*, Macmillan Press, London, pp. 211–233.
16. Oliai, S.E. & B.H. King, 2000. Associative learning in response to color in the parasitoid wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae). *J. Insect Behav.,* 13: 55-69.
17. Papaj, D.R. & R.J. Prokopy, 1989. Ecological and evolutionary aspects of learning in phytophagous insects. *Ann. Rev. Entomol.,* 34: 315-350.
18. Patt, J.M., G.C. Hamilton & J.H. Lashomb, 1999.

Responses of two parasitoid wasps to nectar odors as a function of experience. *Entomol. Exp. Appl.,* 90: 1-8.

19. Sato, M. & K. Takasu, 2000. Food odor learning by both sexes of the pupal parasitoid *Pimpla alboannulatus* Uchida (Hymenoptera: Ichneumonidae). *J. Insect Behav.,* 13(2): 263-272.

20. Smith, B.H., 1993. Merging mechanism and adaptation: an ethological approach to learning and generalization. In: Insect Learning: Ecological and Evolutionary Perspectives (ed.ByACLewis), Chapman and Hall, London, pp. 126-157.

21. Sobhy, I.S., T. Goelen, B. Herrera-Malaver, K.J. Verstrepen, F. Wackers, H. Jacquemyn & B. Lievens, 2019. Associative learning and memory retention of nectar yeast volatiles in a generalist parasitoid. *Ani. Behav.,* 153: 137-146.

22. Stapel, J.O., A.M. Cortesero, C.M. De Moraes, J.H. Tumlinson & W.J. Lewis, 1997. Extrafloral nectar, honeydew, and sucrose effects on searching behaviour and efficiency of *Microplitis crociepes* (Hymenoptera: Braconidae) in cotton. *Environ. Entomol.,* 26: 617-623.

23. Stephens, D.W., 1993. Learning and behavioural ecology: incomplete information and environmental predictability. In: Insect Learning: Ecological and Evolutionary Perspectives (ed.by DR Papaj and AC Lewis), Chapman and Hall, New York, pp. 195–218.

24. Takasu, K. & W.J. Lewis, 1993. Host and food foraging of the parasitoid *Microplitis croceipes*: learning and physiological state effects. *Biol. Cont.,* 3: 70-74.

25. Takasu, K. & W.J. Lewis, 1995. Importance of adult food sources to host searching of the larval parasitoid *Microplitis croceipes. Biol. Cont.,* 5: 25-30.

26. Takasu, K. & W.J. Lewis, 1996. The role of learning in adult food location by the larval parasitoid, *Microplitis croceipes* (Hymenoptera: Braconidae). *J. Insect Behav.,* 9: 265-281.

27. Takasu, K., G.C. Rains & W.J. Lewis, 2007. Comparison of detection ability of learned odours between males and females in the larval parasitoid *Microplitis croceipes. Entomol. Exp. App.,* 122: 247–251.

28. Tumlinson, J.H., T.C.J. Turlings & W.J. Lewis, 1993. Semiochemically mediated foraging behaviour in beneficial parasitic insects. *Arc. Insect Biochem. Physiol.,* 22: 385-391.

29. Turlings, T.C.J., F. Wäckers, L.E.M. Vet, W.J. Lewis & J.H. Tumlinson, 1993. Learning of host finding cues by hymenopterous parasitoids. In: Insect learning: Ecological and evolutionary perspectives. (ed. By DR Papaj and AC Lewis) Chapman and Hall, New York, pp. 51-78.

30. Wäckers, F., D. Olson, G. Rains, F. Lundby & J.E. Haugen, 2011. Boar taint detection using parasitoid biosensors. *J. Food Sci.,* 76(1): 41-47.

31. Wäckers, F.L. & W.J. Lewis, 1994. Olfactory and visual learning and their interaction in host site location by *Microplitis croceipes. Biol. Cont.,* 4: 105-112.

32. Wäckers, F.L., 2004. Assessing the suitability of flowering herbs as parasitoid food sources: flower attractiveness and nectar accessibility. *Biol. Cont.,* 29: 307-314.

33. Wäckers, F.L., C. Bonifay & W.J. Lewis, 2002. Conditioning of appetitive behaviour in the Hymenopteran parasitoid *Microplitis croceipes. Entomol. Exp. Appl.,* 103: 135-138.