

Learning of narcotic odors by a parasitoid

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ABSTRACT

When the parasitic wasp *Microplitis croceipes* experiences odors while feeding on sugar water, it learns to associate the odors with sugar and thereafter exhibits typical food searching behavior in response to the odors. Previous studies have shown that this wasp can be used for detection of the small amount of explosives or other volatile chemicals. In the present study, we examined if this wasp can learn and report narcotic odors. Males of *M. croceipes* were trained to link sugar water with pseudo-narcotic scents that have been used for training narcotic detection dogs, and their behavioral response to the trained odors was observed. The males that had been given either an odor or sugar water did not show any positive response to the odors. However, when the wasps were given a combination of sugar water and either the pseudo-Cocaine, Heroin, LSD or Marihuana, they quickly learned to associate the odors with sugar, and thereafter positively responded to those odors. Our results suggest that this wasp can be used for detection of these narcotics.

Key words : Food searching, Learning, *Microplitis croceipes*, Narcotics, Odors, Parasitic wasp

INTRODUCTION

Parasitic Hymenoptera have ability to learn

to associate odors with hosts, host products or sugars and use those learned odors to search for hosts and/or food [1, 2]. Recent studies

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demonstrated that parasitic wasps can learn a wide variety of odors such as 2,4-DNT, cyclohexanone, cadaverine, and putriscene and detect them at very low concentration, suggesting application of those wasps to odor detection system [3]. Rains *et al.* (2006) has developed the portable odor detection device “Wasp Hound”[4]. This device can detect a target odor by analysing behavioral changes of wasps conditioned the target odor to associate with sugar water. This odor detection system can be used for detecting trace of odors in the similar way as trained canines are used, because this system is flexible to program a variety of odors and as sensitive as trained canines [4, 5]. Although trained wasps is suspected to be used to detect narcotics in airports or ports as trained canines do, learning of narcotic odors by wasps has not been studied yet.

Microplitis croceipes (Hymenoptera: Braconidae) is a larval parasitoid of *Heliothis/Helicoverpa*spp[6]. *Microplitis croceipes* is capable of learning a wide variety of odors associated with hosts or food [1, 3]. After experiencing an odor while feeding on sugar water only a few times, both sexes of *M. croceipes* develop a preference for the odor, and show typical food searching behavior in response to the odor: They walk or fly to ward the odor source, or walk around the odor source area with frequently changing directions [7, 8]. Takasu *et al.* (2007) showed that there was no significant difference in odor learning and detection ability

between sexes of this species [8]. In the present study, we examined whether *M. croceipes* could learn to associate sugar water with pseudo-narcotic odors that are used for training narcotic detection dogs to determine the possibility of use of trained wasps for detection of narcotics.

MATERIALS AND METHODS

Insects

Le & Takasu (2005) showed that a Japanese strain of *H. armigera* is used as an alternative host of *M. croceipes*: *Helicoverpa armigera* was as suitable for parasitism by *M. croceipes* as *H. zea* and *H. virescens* [9]. *Microplitis croceipes* was reared with *H. armigera* in the manner described by Le & Takasu (2005) [9].

Microplitis croceipes adults were kept in a rearing cage (30×30×30 cm), and provided with only water for 2 days after emergence. Then, 2 day-old unfed males were given only an odor, sugar water or a combination of an odor and sugar water for experiments. Rearing and all the experiments were conducted at 25 °C, 50–70 % RH and 16 L:8 D.

Chemicals

The chemicals used for experiments were Sigma pseudoTM Narcotic Scent Cocaine formulation (Canine training aid), Sigma pseudoTM Narcotic Scent Heroin formulation (Canine training aid), Sigma pseudoTM Narcotic Scent Marihuana

formulation (Canine training aid), and Sigma pseudoTM Narcotic Scent LSD formulation (Canine training aid). For each of the chemicals, three different concentrations ranging from 100 μg to 10 mg per 20 μl of dichloromethane were used for conditioning and testing.

Conditioning procedure

Wasps were conditioned odors as described by Takasu *et al.* (2006)[7]. First, 20 ml of dichloromethane containing a certain concentration of a chemical was pipetted on a piece of filter paper (1.5×1.5 cm). The treated filter paper was placed in a Petri dish under ventilation for 1 min to allow the solvent to evaporate. Then, the filter paper was placed in a 250 ml glass jar containing a 3 cm magnetic stirrer. The jar was immediately covered with a sheet of aluminum foil, sealed with a screw lid, and placed on a magnetic stirrer. The stirrer was set at 770 r.p.m for 5 min to distribute the volatile inside the jar. Thereafter, 7 holes were made in circle near the center of the foil. A piece of filter paper (2×2 mm) saturated with 30% sucrose solution was placed in the center of the ringlet of holes.

Individual wasps kept in a test tube (9cm long) were allowed to walk directly from the test tube to the aluminum foil and then to feed on the sucrose solution for 10 s, before being gently removed with forceps. During approaching and feeding, wasps experienced the odor diffusing through the holes. For each wasp, the conditioning session was repeated five times with 30 s

intervals. After the final conditioning session, conditioned wasps were individually kept in a test tube (9 cm long) for 20-30 min before testing.

As controls, wasps were given either sugar water or an odor: Wasps were individually allowed to feed on a sucrose solution (30%) 5 times on the glass jar where no chemical was placed (sugar fed wasps). Other wasps were individually exposed to a narcotic odor 5 times on the glass jar in which a narcotic odor was placed.

Different jars were used for different concentrations to be conditioned and tested. Twelve males were used for each of three treatments. Conditioning and behavioral observations were conducted from 10:00 to 15:00 hours at 25 ± 1 °C, 55-60 % RH and a light intensity of 1200-1300 lux under the fume hood.

Testing procedure

For testing, we used the same glass jar device, but no piece of filter paper saturated with sugar water was placed on the center of a ringlet of holes. The individual wasps kept in a test tube was allowed to walk from the test tube to the foil. The time a wasp remained within a 1-cm radius of the holes was recorded and herein referred to as the responded time. Wasps that remained within the observation radius for less than 10s were regarded responsive ones. All wasps used in our experiments were tested only once, to one odor only, and then

discarded. Three males to five males were tested each day. A total of 12 wasps were tested for each concentration, each compound.

Statistical analysis

The proportions of wasps responding to odors were compared by a post hoc multiple comparison for proportions [10]. Mean times of the wasps responding to odors were compared by one-way ANOVA.

RESULTS

After *M. croceipes* males were given one of 4 pseudo-narcotic odors 5 times, they did not show any food searching behavior in response to the experienced odors, except one male to 40

mg / L of cocaine (Table 1). Neither did males given only sugar water (Table 1). However, when males were given a combination of one of the odors and sugar water 5 times, many of them responded to three tested concentrations of the odors. For all the concentrations of the four odors tested, the percentages of response by males given a combination of an odor and sugar water were significantly greater than those by males given either odors or sugar water (post hoc multiple comparisons in percentages for tests of homogeneity, $P < 0.05$). For males given a combination of an odor and sugar water, the percentages of response did not differ among concentrations regardless of odors (post hoc multiple comparisons in percentages for tests of homogeneity, $P > 0.05$) (Table 1).

Mean response time by males given a

Table 1. Effect of conditioning on behavioral response by *M. croceipes* males to pseudo-narcotic odors

Odor	Concentration per liter	Experience of wasps		
		Odor	Sugar water	Odor + Sugar water
Cocaine	40 mg	8.3 aA	0 aA	83.3 bA
	4 mg	0 aA	0 aA	66.7 bA
	400 mg	0 aA	0 aA	50.0 bA
Heroin	40 mg	0 aA	0 aA	50.0 bA
	4 mg	0 aA	0 aA	58.3 bA
	400 mg	0 aA	0 aA	50.0 bA
Marihuana	40 mg	0 aA	0 aA	41.7 bA
	4 mg	0 aA	0 aA	41.7 bA
	400 mg	0 aA	0 aA	25.0 bA
LSD	40 mg	0 aA	0 aA	33.3 bA
	4 mg	0 aA	0 aA	33.3 bA
	400 mg	0 aA	0 aA	25.0 bA

combination of an odor and sugar water was not different among the same concentrations of different odors and among the different concentrations of the same odors (Table 2).

For the same odors, the percentages followed by the same upper case letter in the same column are not significantly different by post hoc multiple comparison for proportions ($P>0.05$), and the percentages followed by the same lower case letter within a row are not significantly different by post hoc multiple comparison for proportions.

There were no significant differences among the same concentrations of different odors and among different concentrations of the same odors by ANOVA ($P>0.05$).

DISCUSSION

Although control wasps given either only an odor or only sugar water did not respond to pseudo-narcotic odors, wasps that had been conditioned sugar water with an odor responded to the experienced odor. These results suggest that response to these odors is induced by

associative learning. When *M. croceipes* males experience a narcotic odor while feeding on sugar water, they learn to associate the odor with sugar and thereafter respond to the experienced odor. In this associative learning, the sugar is an unconditioned stimuli (US) and the odor a conditioned stimuli (CS). Although naïve wasps normally do not respond to CS, wasps given a combination of US and CS develop a positive response to CS. In both sexes of *M. croceipes*, positive responses to a wide variety of odors are also induced by associative learning [3, 5].

While more than 50% of wasps responded to cocaine and heroin, only 25-40% of them did to marihuana and LSD. The difference may be due to the volatility of these chemicals. With high volatile chemicals such as Methyl benzoate and 3-octanone, wasps quickly learn and all of them respond even to 4 mg/L [8]. However, wasps conditioned with low volatile chemicals such as 2, 4-DNT, respond to the odors at only higher concentrations [11].

Heroin and cocaine have spread dramatically since the 1980s and confiscated drug amounts have increased exponentially. The amount of

Table 2. Response time of *M. croceipes* males that were given a combination of pseudo-narcotic odors and sugar water.

Concentration (per liter)	Mean ± SD (No. of responding wasps)			
	Cocaine	Heroin	Marihuana	LSD
40 mg	27.1 ±14.3(11)	16.8 ± 9.0 (8)	11.4 ± 6.8 (5)	12.6 ± 5.1 (4)
4 mg	17.6 ± 6.7 (8)	14.3 ± 6.2 (7)	11.8 ± 1.9 (7)	12.8 ± 2.1 (5)
400 mg	20.0 ± 9.0 (7)	14.8 ± 4.4 (6)	13.7 ± 3.2 (3)	11.3 ± 3.5 (4)

stimulants confiscated in Japan ranks fifth largest in the world [12]. A 1999 National Household Survey on Drug Abuse reported that approximately 15,000,000 people in the United States use illicit drugs. Narcotic users often develop serious physical, social, and mental health problems that compromise well-being and affect family and friends. To prevent spread of the illegal drugs, tight screenings are conducted at Sea or Airports in US, Japan and other developed countries, using dogs or electronic devices. However, the number of dogs trained for narcotic detection is limited, and electronic devices that have recently developed are still expensive and/or not of sufficient sensitivity. The odor detection system using *M. croceipes* which is not expensive, as sensitive as dogs is considered to be used in situations where dogs or electronic devices are not available [5]. The present study suggests that detection devices using trained *M. croceipes* can be used for narcotics such as cocaine, heroin, marihuana and LSD.

REFERENCES

1. Lewis, W. J. and K. Takasu. 1990. Use of learned odors by a parasitic wasp in accordance with host and food needs. *Nature* 348: 635–636.
2. Vet, L. E. M., W. J. Lewis and R. T. Cardé. 1995. Parasitoid learning. In *Chemical ecology of insect* 2. Cardé, R. T. and W. J. Bell (eds.) pp65–101, Chapman & Hall, New York.
3. Olson, D. M., G. C. Rains, T. Meiner, K. Takasu, M. Tertuliano, J. H. Tumlinson, F. L. Wäckers and W. J. Lewis. 2003. Parasitic wasps learn and report diverse chemicals with unique conditionable behaviors. *Chem. Senses* 28:545–549.
4. Rains, G. C., S. L. Utley and W. J. Lewis. 2006. Behavioral monitoring of trained insects for chemical detection. *Biotech Progress* 22:2–8.
5. Tomberlin J. K., G. C. Rains and M. R. Sanford. 2008. Development of *Microplitis croceipes* as a biological sensor. *Entomol. Exp. Appl.* 128: 249–257.
6. Lewis, W. J. and R.L. Burton. 1970. Rearing *Microplitis croceipes* in the laboratory with *Heliothis zea* as hosts. *J. Econ. Entomol.* 63:656–658.
7. Takasu, K. and W. J. Lewis. 1995. Importance of adult food sources to host searching of the larval parasitoid *Microplitis croceipes*. *Biol. Control.* 5:25–30.
8. Takasu, K., G. C. Rains and W. J. Lewis. 2007. Comparison of detection ability of learned odors between males and females in the larval parasitoid *Microplitis croceipes*. *Entomol. Exp. Appl.* 122:247–251.
9. Le, H. K. and K. Takasu. 2005. *Helicoverpa armigera* as an alternative host of the larval parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae). *Appl. Entomol. Zool.* 40:679–686.
10. Marascuilo L. A. and M. McSweeney. 1977. *Nonparametric and distribution-free methods for the social science*. Brooks/Cole, Monterey, USA.
11. Tomberlin, J. K., M. Tertuliano, G. Rains and W. J. Lewis. 2005. Conditioned *Microplitis croceipes* Cresson (Hymenoptera: Braconidae) detect and respond to 2,4-DNT: development of a biological sensor. *J. Forensic. Sci.* 50:1187–1190.
12. DAPC
<http://www.dapc.or.jp/english/establish.htm>.