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Unrewarding experience with a novel environment modulates olfactory response in the host-searching behavior of parasitic wasps

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Abstract

Generalist insects, which utilize various food resources from various environments, must make decisions to locate resources using novel environmental sensory stimuli when they arrive in a new habitat. In addition to the innate response repertoire, such decision using novel stimuli can include an experience-based process. Here, we showed that the generalist parasitic wasp *Aphidius gifuensis* altered its olfactory responses after exposure to nonnatal habitat environments, i.e., host plants of aphids. In our laboratory experiments, overnight exposure of female wasps to nonnatal broad bean plants reduced their olfactory preference for uninfested bean plants and induced an olfactory preference for host-infested plants over uninfested plants of both broad bean and wheat. The decrease in olfactory preference for uninfested plants was not observed in wasps with overnight exposure to their natal wheat plants. In addition, the olfactory preference for uninfested wheat plants over uninfested bean plants was not observed without previous rewarding experience (oviposition) on the natal wheat plants prior to the overnight exposure to uninfested bean plants. These results suggest that a certain period of unrewarding experience (residency without host finding) on nonnatal plants promoted emigration from the unrewarded environment and increased the wasp's response to olfactory cues from host-infested plants of both species. This study shows negative experience with uninfested plants plays a key role in host-searching behavior and habitat (host plant) selection in the wasp.

Keywords – parasitic wasp; host location; habitat selection; plant volatiles; unrewarding experience

Introduction

Individual organisms use various resources for their survival and reproduction. To find those resources, they use sensory stimuli originating from the resources themselves and from other objects indicating the resources in the environment. The more varied resources in different environments a species uses, the broader the range of sensory stimuli it should experience. The foraging process using environmental information is long and complex in carnivorous insects at higher trophic levels, since their prey and the organisms at trophic levels below the prey can be sources of information (Aartsma et al. 2019). For example, parasitic wasps that target herbivorous insects use visual and olfactory stimuli as cues to find their hosts and preferable microhabitat plants (Vet and Dicke 1992). Parasitic wasps that utilize various hosts of different taxa (generalists) face an enormous variety of olfactory cues from host-infested plants, since volatile cues such as herbivore-induced plant volatiles (HIPVs) are qualitatively and/or quantitatively distinctive in terms of plant species, cultivars, and developmental stages as well as herbivore species and developmental stages (Takabayashi and Dicke 1996; Dicke 1999; Sabelis et al. 2007). The ability of parasitic wasps to respond to olfactory cues is partly innate, but later in life, such abilities are acquired and modified through experience (Turlings et al. 1993; Vet et al. 1995; Takabayashi et al. 2006; Giunti et al. 2015; Kaiser et al. 2017). In many parasitic wasp species, individuals disperse from the habitat where they developed (natal habitat) and spread to different nonnatal habitats, using different hosts on different plants (Steidle and van Loon 2003). After the arrival of wasps on a less familiar plant, they are expected to modulate their response to the less familiar sensory stimuli through experience for efficient habitat evaluation and host searching.

When parasitic wasps newly arrive in nonnatal habitats, their first contact is with plants that are not inhabited by host insects. Odor from uninfested plants may play an important role in parasitoid foraging, as habitat cue (Webster & Cardé 2017) or as background volatiles which make it more difficult for parasitoids to filter out the odor of the host infested plants (Schröder & Hilker 2008). Mumm and Hilker (2005) showed that the odor of host-infested plants (i.e., an increased amount of (*E*)- β -farnesene induced by host oviposition) alone did not attract the egg parasitoid *Chrysonotomyia ruforum* but, when mixed with the odor of uninfested plants, did attract them. These studies respectively demonstrate that the odors of uninfested plants play a key role in their host-searching behavior of those wasps. The effects of odors from uninfested plants on recognition of odor sources

by wasps may appear at the level of the receptors in the sensory organs (Schröder and Hilker 2008) or at the level of memory (Kraemer and Golding 1997). . Unrewarding experience with nonhost-infested plants was shown to affect the olfactory preferences of parasitic wasps (Steven et al. 2019). The parasitic wasp *Cotesia kariyai* showed reduced rates of taking-off behavior, increased duration until landing, reduced landing rates on the nonhost (tobacco cutworm)-infested maize plants, and reduced searching duration on both host (common army worm)- and the nonhost-infested plants after the experience of contact with the nonhost on the nonhost-infested plants (Steven et al. 2019). Unrewarding search on uninfested plants may similarly alter the response to uninfested plants and thus mediate parasitoid behavior in their natal as wells as in unfamiliar habitats. However, the effect of the unrewarding experience of uninfested plants has been less extensively investigated.

Aphidius gifuensis Ashmead (Hymenoptera: Braconidae) is a parasitic wasp inhabiting eastern Asia (Takada 1992). This species is a generalist species that attacks a broad range of aphid species spanning multiple taxa (Takada 2002; Ohta and Honda 2010) and is being used as a biological control agent (e.g., Wei et al. 2003; Ohta and Ohtaishi 2005; Yang et al. 2009; Pan and Liu 2014). A previous study showed that *A. gifuensis* tends to fly out spontaneously from its natal habitat (wheat plants) to a neighboring nonnatal habitat (broad bean plants) in response to general visual plant stimuli (green color or brightness) after encountering host aphids in the natal habitat (Takemoto 2016). Naïve wasps as well as wasps that either experienced uninfested or host-infested wheat alone did not prefer volatiles from host-infested wheat plants over volatiles from uninfested plants and preferred volatiles from uninfested bean plants over volatiles from host-infested bean plants. Only complete experience with uninfested and infested wheat plants resulted in a preference for volatiles from host-infested wheat plants over volatiles from uninfested wheat. Volatiles from uninfested and volatiles from host-infested bean plants were equally attractive for wasps with complete experience on uninfested and host-infested wheat plants (Takemoto 2016). Therefore, the ability of female *A. gifuensis* wasps to use host-searching cues on a nonnatal bean plant is limited when they newly arrive at the habitat.

In this study, to examine whether prolonged exposure to natal or nonnatal plants would differentially affect the olfactory responses of wasps to uninfested plants, we compared the responses of female wasps under two different conditions: (1) those exposed overnight to natal wheat plants without host aphids (wheat-exposed wasps) and (2) those exposed to nonnatal bean plants without host aphids (bean-exposed wasps). In experiment 1, we predicted that the preference of bean-exposed wasps for wheat plants would be high because those wasps would have experienced an unrewarded search on bean plants. The preference of wheat-exposed wasps for bean plants would also be high because oviposition in another host/plant system is broadly observed in the wasp *A. gifuensis* (Ohta and

Honda 2010). In experiment 2, we predicted that the choice of wasps between host-infested and uninfested bean plants would not be the same between the wheat-exposed and bean-exposed wasps. Specifically, we hypothesized that the bean-exposed wasps would show a preference for host-infested bean plants over uninfested bean plants because such a preference might promote host finding by wasps in an environment of novel plants, as empirically observed (Ohta and Honda 2010).

Materials and Methods

Plants and insects

Four grams of *Triticum aestivum* Linnaeus cv Norin 61 (Poneceae) wheat seeds, equaling approximately 100 seeds, were germinated in a vinyl pot (upper internal diameter 75 mm and lower internal diameter 55 mm×height 66 mm) filled with vermiculite. Ten to 14 days after germination, the wheat seedlings (approximately 50 seedlings) were used for insect rearing and experimentation. Broad bean plants, *Vicia faba* Linnaeus cv Nintoku Issun (Fabaceae), were germinated on vermiculite and transplanted individually into vinyl pots. Three weeks after germination, bean seedlings with two or three pairs of extended leaves were used for insect rearing and experimentation. In total, over four hundred pots with one plant each were used for experimentation. The plants were grown in a climate-controlled room (22 ± 2 °C, 14 L:8 D, 20-40% relative humidity).

A colony of *A. gifuensis* was provided by Izumi Ohta in 2012 and maintained on *Sitobion akebiae* Shinji (Hemiptera: Aphididae) on wheat plants. The population originated from *Myzus persicae* mummies on eggplants in Nangoku City, Kochi Prefecture, Japan, in April 2004. The alternative host aphids *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae) were collected in Shizuoka, Japan, and maintained on broad bean (*V. faba*) plants. For the test wasp preparation, 100 adult *S. akebiae* aphids were transferred to a pot of wheat plants, and 3 or 4 days later, the wheat plant-host aphid complex was exposed to 30 adult female *A. gifuensis* wasps. Eleven to twelve days after exposure, the resulting mummies were gently collected with forceps and used in the experiments. For the preparation of odor sources, broad bean plants infested for 3-4 days with 50 adult pea aphids (*A. pisum*) were also prepared.

Treatment of wasps

An overview of the wasp preparation (conditioning) procedure is shown in Fig. 1, and the details are as follows. Four hundred mummies (parasitized aphid bodies containing wasp pupae) were placed next to 2 pots of wheat plants in a plastic cage to expose the wasps to uninfested wheat plants from

emergence to the early adult stage, thereby imitating wasp emergence in the natal habitat. One hundred twenty emerged adult females, aged one to four days after emergence, were collected and released into another plastic cage containing 2 pots of wheat plants infested with 100 *S. akebiae* adults for 3 or 4 days. In this scenario, they encountered (experienced) the hosts in the natal habitat. Thus, among 400 mummies, 120 female wasps aged 1-4 days were collected for the treatments, and the remaining 280 were 0-day-old females (emerged on the day of collection), unemerged mummies, male wasps, and remaining female wasps not needed for experiments. We used only females aged 1-4 days, rather than 0 days, because most of the females are expected to copulate with males within a day (24 hours) after emergence (Wei et al. 2003). After 30 min of experience of the infested natal wheat plants, 90 wasps were collected from the plants (the other 30 out of 120 were not on plants at the time) and transferred into another plastic cage, where they were maintained overnight with either 2 pots of uninfested wheat plants (for the wheat-exposed treatment) or 2 pots of uninfested bean plants (for the bean-exposed treatment). Thirty minutes of exposure is predicted to be long enough for a wasp to lay an egg on an aphid, as a female wasp with no oviposition experience will lay an egg immediately after contact with an aphid (Takemoto 2016; Du et al. 1997). The wheat-exposed wasps had experienced 30 min of exposure to aphids on natal wheat plants followed by overnight exposure to natal wheat, and the bean-exposed wasps had experienced 30 min of exposure to aphids on natal wheat plants followed by overnight exposure to nonnatal bean plants. Throughout the treatment procedure, the wasps had access to 50% (w/v) aqueous honey solution, which was also placed in the cages. This procedure was repeated many times (35 times) to obtain enough samples (150 wasps) for each test category.

Olfactory preferences

The olfactometer consisted of 2 odor source containers and a choice arena constructed of a Petri dish attached to the bottom of the odor source container, which was modified as described in a previous study (Takemoto 2016). The choice arena and the odor source containers were connected to holes (25 mm) covered with trap tubes. Purified air was introduced in the odor source container at a rate of 100 ml/min. Twenty-five to thirty female wasps were collected in a cartridge, which was attached to the choice arena for release. We observed the wasps shortly after release and observed no contact between wasps. This species is a tiny solitary wasp, and the choice arena is large enough for them to choose their habitats without contacting other wasps. Therefore, we expect no effect of mass release in this experiment. One hour after the release, the wasps in each trap tube were counted. Six replicates were conducted on different days for each combination of odor sources using different plant pots and different wasps. Each wasp was tested only once. The order of test combinations was determined arbitrarily.

In experiment 1, the following 3 combinations of odor sources were tested:

- (a) air (wet paper towel) vs. uninfested wheat plants;
- (b) air (wet paper towel) vs. uninfested bean plants; and
- (c) uninfested wheat plants vs. uninfested bean plants.

In addition, the choice test of (c) uninfested wheat plants vs. uninfested bean plants using bean-exposed wasps without previous rewarding experiences was also conducted to investigate whether the behavioral change during prolonged exposure treatment in nonnatal bean-exposed wasps is affected by the previous rewarding experiences.

In experiment 2, the following 3 combinations, including a total of four odor sources, were tested:

- (a) uninfested wheat plants vs. host-infested wheat plants
- (b) uninfested bean plants vs. host-infested bean plants
- (c) host-infested wheat plants vs. host-infested bean plants

Statistics

All statistical analysis was performed with R ver. 3.5.2 (R developmental core team, 2018). The total number of wasps counted for each odor source was compared using a chi-squared test ($\alpha = 0.05, 0.01, 0.001$) to test for an overall difference from the null hypothesis of no preference (50:50 choice). Wasps that did not make choices were not included in the statistical analyses. In order to test whether the preference differed between wasps receiving different overnight exposure treatments, the responses of wasps within each odor source combination were analyzed using a generalized linear mixed model (GLMM) with a binomial distribution and a logit-link function (glmmML function in glmmML package). The combinations of the number of parasitoids that chose source 1 and the number of parasitoids that chose source 2 in each replicate (odor sources) were made with the cbind function in R and used as the response variable, and the treatment of overnight exposure (to wheat or bean plants) was used as the explanatory variable. Replicate (odor sources) was included as the random effect. Inferences regarding the treatment were made based on a likelihood ratio test using a parametric bootstrap simulation with 10000 replicates, which compares the deviance of the model with the effect of treatment and the model without it (Faraway 2016).

Results

Experiment 1.

The wheat-exposed wasps were attracted to uninfested plants. They preferred wheat and bean over air (air 39, uninfested wheat plants 60, $n = 99$, $\chi^2 = 4.455$, $df = 1$, $p = 0.035$; air 31, uninfested bean plants 54, $n = 85$, $\chi^2 = 6.224$, $df = 1$, $p = 0.013$) (Fig. 2a, 2b) and were equally attracted to uninfested

wheat and bean (uninfested wheat plants 55, uninfested bean plants 45, $n = 100$, $\chi^2 = 1.000$, $df = 1$, $p = 0.317$) (Fig. 2c). Bean-exposed wasps on the contrary did not prefer uninfested wheat or bean over air (air 35, uninfested wheat plants 50, $n = 85$, $\chi^2 = 2.647$, $df = 1$, $p = 0.104$; air 55, uninfested bean plants 44, $n = 99$, $\chi^2 = 1.222$, $df = 1$, $p = 0.269$) (Fig. 2a, 2b) but preferred uninfested wheat over uninfested bean (uninfested wheat plants 52, uninfested bean plants 27, $n = 79$, $\chi^2 = 7.911$, $df = 1$, $p = 0.005$) (Fig. 2c). This preference for wheat over bean was not observed in wheat-exposed wasps, as mentioned above (Fig. 2c), and in bean-exposed wasps without previous rewarding experience on wheat system (uninfested wheat plants 44, uninfested bean plants 49, $n = 93$, $\chi^2 = 0.269$, $df = 1$, $p = 0.604$) (Fig. 3). Previous rewarding experience altered the choice by bean-exposed wasps (GLMM: δ deviance = 4.5574, $p = 0.023$).

Different overnight exposure treatments (to wheat or bean) did not influence parasitoid choice between air and uninfested wheat (GLMM: δ deviance = 0.0703, $p = 0.787$) (Fig. 2a) or between host-infested wheat and uninfested bean (GLMM: δ deviance = 1.3726, $p = 0.224$) (Fig. 2c), but altered the choice between air and uninfested bean (GLMM: δ deviance = 4.9888, $p = 0.019$), (Fig. 2b). This result suggested that wheat-exposed wasps were more strongly attracted to uninfested bean plants than bean-exposed wasps.

Experiment 2.

The wheat-exposed wasps did not prefer host-infested wheat over uninfested wheat (uninfested wheat plants 35, host-infested wheat plants 43, $n = 78$, $\chi^2 = 0.821$, $df = 1$, $p = 0.365$) (Fig. 4a), but preferred host-infested bean over uninfested bean (uninfested bean plants 27, host-infested bean plants 48, $n = 75$, $\chi^2 = 5.880$, $df = 1$, $p = 0.015$) (Fig. 4b), while they were equally attracted to host-infested wheat and bean (host-infested wheat plants 42, host-infested bean plants 57, $n = 99$, $\chi^2 = 2.273$, $df = 1$, $p = 0.132$) (Fig. 4c). Bean-exposed wasps preferred host-infested plants over uninfested plants (uninfested wheat plants 21, host-infested wheat plants 64, $n = 85$, $\chi^2 = 21.753$, $df = 1$, $P < 0.001$; uninfested bean plants 36, host-infested bean plants 64; $n = 100$, $\chi^2 = 7.840$, $df = 1$, $p = 0.005$) (Fig. 4a, 4b) and were equally attracted to host-infested wheat and bean (host-infested wheat plants 47, host-infested bean plants 33; $n = 80$, $\chi^2 = 2.450$, $df = 1$, $p = 0.118$) (Fig. 4c).

Overnight exposure to uninfested bean or wheat plants did not alter the preference of wasps for host-infested bean plants over uninfested bean plants (GLMM: δ deviance = 0.0009, $p = 0.981$) (Fig. 4b), but wheat-exposed wasps were significantly less attracted to host infested wheat plants (GLMM: δ deviance = 4.4390, $p = 0.028$) (Fig. 4a) and chose more often host-infested bean plants over host-infested wheat plants (GLMM: δ deviance = 4.7316, $p = 0.024$) (Fig. 4c) compared to bean-exposed wasps.

Discussion

Information use by parasitoids is context dependent and influenced both by rewarding experiences on host-infested plants and by non-rewarding experience on uninfested plants. Depending on previous rewarding and non-rewarding experiences, parasitoid responses to the same volatile cues differed, indicating that these cues are interpreted differently in different contexts. In the wasps with oviposition experience in the natal habitat wheat, the prolonged negative experience with uninfested bean plants (nonnatal habitat) resulted in no attraction to uninfested wheat or bean plants (compared to pure air) and in a general attraction towards host-infested plants (wheat and bean). The bean-exposed wasps with a host experience in the natal habitat significantly preferred uninfested wheat over bean plants, while those bean-exposed wasps without host experience in the natal habitat did not prefer uninfested wheat over bean plants. The negative overnight experience with uninfested wheat plants (natal habitat) resulted in a general attraction towards uninfested plants, but in a low response towards volatiles from host-infested wheat plants (no significant preference for host-infested over uninfested wheat), while volatiles from host-infested bean plants were attractive.

The bean-exposed wasps had a positive experience in their natal habitat, followed by a negative experience in the nonnatal habitat. This experience may be interpreted by the parasitoids as follows: they had been in a high-quality habitat with hosts (wheat), but currently are in a lower-quality habitat without hosts (bean). Orienting towards volatiles from host-infested plants and ignoring volatiles from uninfested bean, and to a lesser extent, from uninfested wheat, may guide them towards a novel habitat that contains hosts. Interestingly, infested bean was found to be repellent to wasps without complete experience on uninfested and infested wheat (Takemoto 2016).

For the bean-exposed wasps without host experience in their natal habitat wheat, both habitats appear to be of low-quality. These wasps should ignore cues from uninfested bean plants, which would keep them in their current habitat and from uninfested wheat which may lead them back to their low-quality natal habitat in which they did not experience any hosts. For the bean-exposed wasps with a positive experience in the natal habitat, a return to natal habitat guided by volatiles from uninfested wheat may not be favorable, due to the risk of superparasitism, inbreeding etc., but the chances to (re-) encounter hosts in this habitat is recognized higher than in the current habitat, which may explain their preference of uninfested wheat over uninfested bean volatiles.

The wheat-exposed wasps first experienced uninfested wheat plants in their natal habitat, followed by a positive experience on host-infested wheat, again followed by a prolonged negative experience

in the same habitat (wheat). This experience may be interpreted the parasitoid as if they are in a medium-quality habitat, which contains hosts at a low density. It may thus be an adaptive strategy to follow cues from host-infested or uninfested plants in a different habitat (bean) or to follow volatiles from uninfested wheat plants to disperse in the natal habitat. The weak response towards volatiles from infested wheat plants may reduce the risk to re-encounter the plant on which the aphids were already parasitized. It was previously shown that only those parasitoids, which had complete experience with both uninfested and host-infested wheat preferred volatiles from host-infested wheat plants over volatiles from uninfested wheat (Takemoto 2016), while the current study shows that this preferences vanishes after a prolonged negative experience with uninfested wheat, stressing the plasticity of the responses to volatiles from host-infested plants in this generalist parasitoid.

Strikingly, the response of this parasitoid species to volatiles from host-infested bean plants strongly depends on their experience with uninfested and host-infested wheat plants (Takemoto 2016) and with uninfested bean plants, ranging from repellence by volatiles from host-infested bean (naïve wasps and wasps with incomplete experience on host-infested or uninfested wheat plants) over no preference between volatiles from host-infested and uninfested bean plants (wasps with complete experience on uninfested and host-infested wheat plants) to attraction to volatiles from host-infested bean plants (complete experience on wheat plants followed by overnight experience with uninfested bean plants). Such plasticity of the responses to volatiles from nonnatal host-infested plants might be involved in the host utilization of this parasitic wasps on plural host-plant systems observed in a banker-plant forming system (Ohta and Honda 2010).

Changes in olfactory preference after unrewarding experiences have been reported in parasitic wasps for both natural substrates (Papaj et al. 1994; Vet et al. 1998) and artificial odors (Iizuka and Takasu 1998; Takasu and Lewis 2003). In an egg-larval parasitoid, *Ascogaster reticulata* Watanabe (Hymenoptera: Braconidae), sequential alternation between rewarding and unrewarding experiences three times at 15-min intervals allowed the wasps to discriminate odor from closely related *Camellia* plants (Seino and Kainoh 2008). These studies explained the possible function of unrewarding experiences. Furthermore, it has been assumed that coupling of rewarding and unrewarding experiences plays an important role in the host-finding process in complex odor environments (Wäschke et al. 2013). In natural environments, odors of host-infested plants are provided to the wasps within odors of the surrounding uninfested plants. Background odors can contain repellent compounds or compounds that mask the perception of ‘resource-indicating odours’ (Schröder and Hilker 2008; Beyaert and Hilker 2014). Several studies have reported the role of repellent compounds in background odors in host/nonhost segregation (Perfecto and Vet 2003; Zhang and Schlyter 2004; Soler et al. 2007; Bruce and Pickett 2011). For example, the response to host plants

was increased by neighboring nonhost plants in *Cotesia glomerata* L. (Hymenoptera: Braconidae) (Perfecto and Vet 2003). However, an increase in olfactory responses to host-infested plants does not always increase actual host-searching efficiency. oviposition experience on a host-infested leaf decreased foraging efficiency due to more frequent visits of non-host-infested plants. The parasitic wasp *Cotesia glomerata* took longer to reach host-infested plants in presence with nonhost-infested plants after oviposition experience on the host-infested plants (Vosteen et al. 2019). Our current study showed that a negative unrewarding experience of uninfested plants by female *A. gifuensis* wasps increased their responsiveness to the volatiles from host-infested plants in a nonnatal habitat. The role of its unrewarding experience in actual host-searching efficiency after dispersal from a natal habitat need to be investigated.

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Author contributions

H.T. designed and performed the experiment, H.T. and J.Y. interpreted the results and wrote the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Figure captions

Fig. 1 Overview of wasp preparation (Experiments 1 and 2).

Fig. 2 Olfactory response of female *A. gifuensis* wasps exposed overnight to natal wheat plants or nonnatal bean plants: (a) air versus uninfested wheat plants, (b) air versus uninfested bean plants, (c) uninfested wheat plants versus uninfested bean plants (Experiment 1). * $P < 0.05$, ** $P < 0.01$, ns = not significant (chi-squared test for significance of each choice test, GLMM for the effect of wasp treatments in the two-choice tests).

Fig. 3 Olfactory response of female *A. gifuensis* wasps without previous exposure to the plant-host complexes (rewarding experience). ns = not significant (chi-squared test).

Fig. 4 Olfactory response of female *A. gifuensis* wasps exposed overnight to natal wheat or nonnatal bean plants: (a) uninfested wheat plants versus host-infested wheat plants (b) uninfested bean plants versus host-infested bean plants (c) host-infested wheat plants versus host-infested bean plants (Experiment 2). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant (chi-squared test for significance of each choice test, GLMM for the effect of wasp treatments in the two-choice tests).

Supplementary table: Wasp counts in all choice assays.

Experiment 1	treatment of wasps	odor source 1	wasp counts	total wasp counts	odor source 2	wasp counts	total wasp counts	n	chi-squared	p value						
wheat-exposed wasps		Air	2	39	uninfested wheat	9	60	99	4.455	0.035						
			9			17										
			4			8										
			6			9										
			9			10										
			9			7										
			5			31					uninfested bean	7	54	85	6.224	0.013
			3									10				
			1									10				
			8									10				
7	13															
7	4															
13	55	uninfested bean	6	45	100		1.000	0.317								
5			11													
5			9													
9			7													
12			6													
11			6													
bean-exposed wasps			Air						3	35	uninfested wheat	10	50	85	2.647	0.104
									7			6				

		5			15				
		9			4				
		7			7				
		4			8				
	Air	15	55	uninfested bean	7	44	99	1.222	0.269
		6			11				
		11			6				
		7			5				
		6			6				
		10			9				
	uninfested wheat	8	52	uninfested bean	3	27	79	7.911	0.005
		9			4				
		15			2				
		9			4				
		5			8				
		6			6				
bean-exposed wasps without previous exposure to plant-host complexes	uninfested wheat plants	6	44	uninfested bean plants	11	49	93	0.269	0.604
		9			8				
		10			7				
		9			8				
		5			9				
		5			6				

Experiment 2	treatment of wasps	odor source 1	wasp counts	total wasp counts	odor source 2	wasp counts	total wasp counts	n	chi-squared	p value
wheat-exposed wasps		uninfested wheat	7	35	host-infested wheat	4	43	78	0.821	0.365
			6			7				
			7			8				
			6			8				
			5			6				
		4	10							
		uninfested bean	host-infested bean	9	27	4	48	75	5.880	0.015
				5		11				
				5		6				
				2		10				
3	8									
3	9									
host-infested wheat	host-infested bean	7	42	8	57	99	2.273	0.132		
		12		12						
		5		7						
		6		12						
		8		8						
4	10									
bean-exposed wasps	uninfested wheat	0	21	host-infested wheat	13	64	85	21.753	< 0.001	
		6			5					
		8			9					

	3			16				
	2			9				
	2			12				
uninfested bean	9	36		14	64	100	7.840	0.005
	5		host-infested bean	15				
	2			10				
	6			5				
	5			13				
	9			7				
host-infested wheat	10	47		8	33	80	2.450	0.118
	10		host-infested bean	3				
	5			8				
	6			7				
	7			4				
	9			3				

Fig. 1

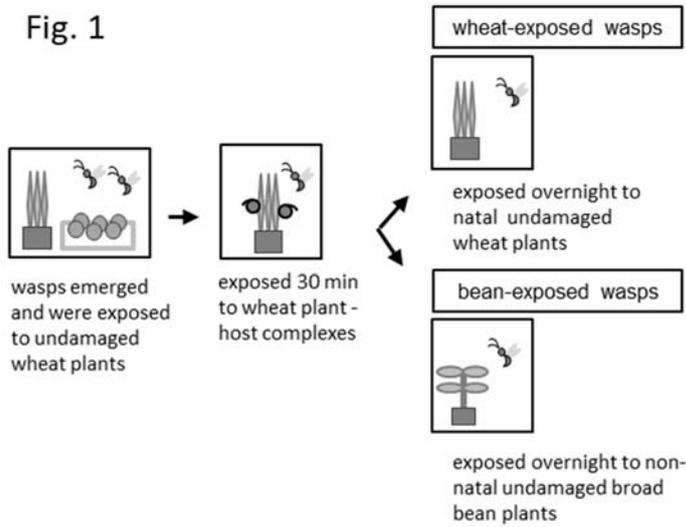


Fig. 2

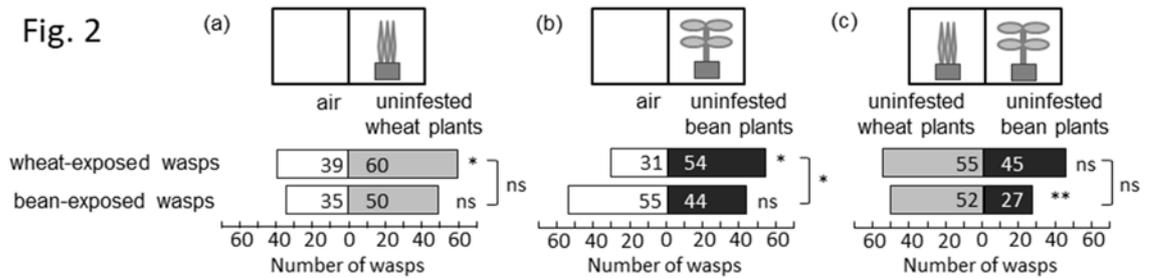


Fig 3

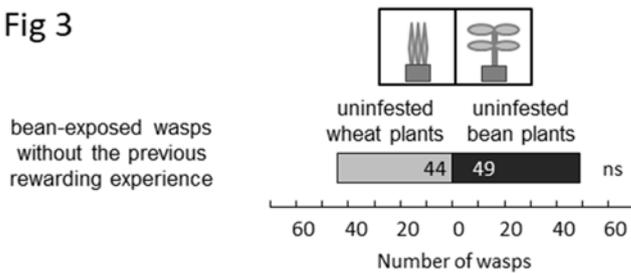


Fig. 4

