

1 OVIPOSITION EXPERIENCE OF PARASITOID WASPS WITH NONHOST
2 LARVAE AFFECTS THEIR OLFACTORY AND CONTACT-BEHAVIORAL
3 RESPONSES TOWARD HOST- AND NONHOST-INFESTED PLANTS
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20 **Acknowledgements** We are grateful to the Japan Ministry of Education, Culture, Sports
21 and Technology (MEXT) for giving SS a Scholarship (period: Oct 2011 to March 2013)
22 during his stay as a student of the Teacher Training Program. This study was supported
23 in part by grants for scientific research (A) from MEXT.
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Abstract - In nature, parasitoid wasps encounter and sometimes show oviposition behavior to nonhost species. However, little is known about the effect of such negative incidences on their subsequent host-searching behavior. We tested this effect in a tritrophic system of maize plants (*Zea mays*), common armyworms (hosts), tobacco cutworms (nonhosts), and parasitoid wasps, *Cotesia kariyai*. We used oviposition inexperienced *C. kariyai* and *negative-experienced* individuals that had expressed oviposition behavior toward nonhosts on nonhost-infested maize leaves. We first observed the olfactory behavior of *C. kariyai* to volatiles from host-infested plants or nonhost-infested plants in a wind tunnel. Negative-experienced wasps showed significantly lower rates of taking-off behavior (Step-1), significantly longer duration until landing (Step-2), and lower rates of landing behavior (Step-3) toward nonhost-infested plants than inexperienced wasps, however, the negative-experience did not affect these three steps toward host-infested plants. We concluded that the negative experience reduced the selectivity of the olfactory response in wasps to nonhost-infested plants. The chemical analyses suggested that the wasps associated (Z)-3-hexenyl acetate, a compound that was emitted more in nonhost-infested plants, with the negative experience, and reduced their response to nonhost-infested plants. Furthermore, we observed that the searching duration of wasps on either nonhost- or host-infested plants (Step-4) was reduced on both plant types after the negative experiences. Therefore, the negative experience in Step-4 would be nonadaptive for wasps on host-infested plants. Our study indicated that the density (i.e., possible encounters) of nonhost species as well as that of host species in the field should be considered when assessing the host-searching behavior of parasitoid wasps.

Key Words - Tritrophic interaction, negative experience, host-finding behavior, (Z)-3-hexenyl acetate.

INTRODUCTION

In nature, nonhosts and hosts of parasitoid wasp species coexist in a plant community or even on the same plant. Thus, it is likely that wasps encounter nonhosts, and consequently, may show oviposition behavior to nonhosts (e.g., Takabayashi and Takahashi 1990). However, the effects of such “misunderstandings” on the subsequent host-searching behavior of parasitoid wasps is not well understood. Although the conditions were unnatural, Takasu and Lewis (2003) demonstrated that the flight response of parasitoid wasps *Microplitis croceipes* to vanilla odor was positively affected by their previous oviposition experience in a host (*Helicoverpa zea* larva) in the presence of vanilla odor, but their response was not affected by their previous oviposition experience in a nonhost (*Spodoptera exigua* larva) in the presence of vanilla odor.

Carnivorous natural enemies such as parasitoid wasps and other carnivorous species respond to “herbivory-induced plant volatiles” (HIPVs) that are emitted from plants infested with their hosts/prey (Arimura et al. 2009; Hare 2011; McCormick et al. 2012; Turlings and Erb 2018). The responses by natural enemies to HIPVs are either due to their innate olfactory preferences (e.g., Geervliet et al. 1996; Shiojiri et al. 2000) or their development of appropriate responses to HIPVs via olfactory learning (e.g., Takemoto et al. 2012), or both (van Oudenhoove 2017 for review; Yoneya et al. 2018). Herein, we studied whether the oviposition behavior of parasitoid wasps to nonhosts on nonhost-infested plants affected their subsequent response to host-HIPVs and nonhost-HIPVs.

In the present study, we used a tritrophic system of maize plants (*Zea mays*: Poaceae), hosts (*Mythimna separata*, Lepidoptera: Noctuidae) (common armyworms: CAWs), nonhosts (*Spodoptera litura*, Lepidoptera: Noctuidae) (tobacco cutworms: TCWs), and specialist parasitoid wasps (*Cotesia kariyai*, Hymenoptera: Braconidae). CAWs use plants from the family Poaceae as food sources, while TCWs use a wider range of plant families, including Poaceae, as food sources. Plants infested with either CAWs or TCWs have been shown to emit respective HIPVs (Choh et al. 2013; Takabayashi et al. 1995). *Cotesia kariyai* females have been shown to be attracted to host (CAWs)-HIPVs in their search for host-infested plants (Takabayashi et al. 1995). When encountering nonhosts (e.g., TCWs) in a petri dish, *C. kariyai* females show

oviposition behavior towards them and lay fewer eggs than when encountering CAWs (Y. Kainoh, unpublished data).

In this study, we aimed to answer the following two questions: (1) are *C. kariyai* females attracted to HIPVs emitted from maize plants infested with nonhosts (TCWs), and (2) if so, how does the experience of oviposition in nonhosts affect their subsequent olfactory responses to host- and nonhost-HIPVs, and their antennal host searching behavior on host- and nonhost-infested plants? We also conducted chemical analyses of CAW-HIPVs and TCW-HIPVs to assess the possible volatile cue(s) related to the nonhost-experience that affected the olfactory responses of *C. kariyai*.

METHODS AND MATERIALS

Insects and Plants. The laboratory CAW and TCW larval colonies were reared on an artificial diet [Silkmate[®] 2(s), Nosan Corporation, Yokohama, Japan] in a climate-controlled room [25 ± 1 °C, $60 \pm 10\%$ relative humidity (RH), 16 light (L):8 dark (D)]. To collect eggs, ~30 adult moths of one species were housed per container and a piece of paraffin paper, folded in an accordion manner, was placed in each container ($35 \times 21 \times 23$ cm, length \times depth \times height) as an oviposition substrate. Paraffin papers with deposited eggs were collected daily. Newly emerged larvae of CAWs and TCWs were maintained in plastic Petri dishes (14.5×2.5 cm, diameter \times height) in early stages and in plastic containers ($28 \times 21 \times 10$ cm, L \times D \times H) in later stages until pupation.

The laboratory colony of *C. kariyai* was maintained under the same conditions used for rearing herbivores. Fourth stadium CAW individuals were offered to 2~3-day-old female *C. kariyai* individuals in small plastic Petri dishes (5.5×2.0 cm, D \times H) for parasitization. Soon after the attack, the host larvae were collected and reared with Silkmate[®] in plastic Petri dishes (14.5×2.5 cm, D \times H). The containers were checked daily until egression, which occurred ~10 d after the incidence of parasitism. A cluster of cocoons of *C. kariyai* was collected and placed in a transparent plastic container ($29 \times 18 \times 17$ cm, L \times D \times H) until emergence of the adults. Cotton wool moistened with honey droplets was provided as food for the parasitic wasps. Mating occurred immediately after emergence. All experimental parasitoid females were 3-day-old, mated, and well-fed individuals.

Maize seedlings (*Z. mays* L., Honey-Bantam Peter 619; Sakata Seed Co.,

Kanagawa, Japan) were grown separately in plastic pots (5.5 × 7.0 cm, D × H) in an incubator (25 ± 1 °C, 60 ± 10% RH, 1500 lx, 16 L:8 D photoperiod). Nine-day-old seedlings were then transplanted to bigger plastic pots (11 × 9 cm, D × H) and reared in a greenhouse (25 ± 1 °C, 14 L:10 D, 60 ± 10% RH). Plants were watered daily and 15-day-old maize plants (~30 cm in height) were used for all experiments.

For the bioassays, CAWs, TCWs, *C. kariyai*, and maize plants were reared in the Laboratory of Applied Entomology and Zoology, University of Tsukuba, Japan. For the chemical analyses, CAWs and TCWs were reared in the Center for Ecological Research, Kyoto University.

Wind Tunnel Bioassay. Behavioral responses of *C. kariyai* females were observed in a wind tunnel (30 × 30 × 150 cm) (25–30 cm/s wind speed, 25 ± 1 °C, 60 ± 10% RH, 16 L:8 D, ~900 lx light intensity) from 13:00 to 16:00 on three or four different days. In each experiment, an individual *C. kariyai* female was released from a platform (15 cm in height), which was placed 30 cm downwind from the odor source. *Cotesia kariyai* females were given 5 min to fly to the target plants. If *C. kariyai* females did not reach the plants within the 5 min period, the wasps were considered as nonresponsive individuals and were not included in the statistical analyses.

Infested plants were prepared in a mesh cage (50 × 50 × 90 cm). The plastic pot (11 × 9 cm, D × H) and the soil surface were covered with aluminum foil. We prepared host-infested plants (damaged by five CAW larvae) and nonhost-infested plants (damaged by five TCW larvae). Damage area of each treatment was adjusted to be ~30% of the total leaf area. The duration of feeding was 19 h (from 18:00 to 13:00). Feces on the surface of aluminum foil were carefully removed with a fine brush prior to the experiments.

Cotesia kariyai is a diurnal wasp species (Sato et al. 1983) and, therefore, oviposits into hosts or nonhosts during the daytime. Based on this, the preparation of experienced *C. kariyai* females were as follows. Three detached maize leaves were placed in a Petri dish (14.5 × 2.5 cm, D × H) and 20 fourth-instar TCW larvae were allowed to feed on the leaves for 1 h under light conditions. An individual *C. kariyai* female was then released into the dish and allowed to search for a nonhost that was feeding on one of the maize leaves. Once a wasp encountered a TCW larva, it expressed oviposition behavior towards the larva. Attacked larvae were removed, and the wasps

were collected as experienced wasps. Groups of experienced wasps were prepared ~3 h before the experiments.

The following steps were measured in each experiment: Step-1: the relative ratio (%) of taking-off; Step-2: the duration from taking-off to landing; Step-3: the relative ratio (%) of landing, and Step-4: the duration of searching on the maize plants. The experiments were repeated 10 times per day using same odor sources. In total, 30 (the response of inexperienced female to host-infested plants) and 40 individuals (others) were tested in 3 or 4 different experimental days.

Chemical Analyses. We analyzed the headspace volatiles of potted maize plants that had been infested with five fourth-stadium larvae (either CAW or TCW) for 19 h. Plants were the same size as those used for the wind tunnel experiments. During the volatile collection, the plant-growing cups and the soil surface were covered with aluminum foil to prevent the collection of non-target volatiles from these sources. Infested plants were used after the removal of feces with a fine brush. We also analyzed volatiles from three detached maize leaves that had been infested with 20 fourth-instar TCW larvae for 1 h.

Volatile collections were performed in a climate-controlled room ($25 \pm 2^\circ\text{C}$, 50–70% RH). A piece of filter paper infiltrated with 1 μL of hexane solution of tridecane (0.1 $\mu\text{g}/\mu\text{L}$) was used as an internal standard. We placed an odor source and the internal standard in a glass bottle (2 L) that had two holes connected to two nozzles. One nozzle was connected to a pure air cylinder and the other nozzle was connected to a glass tube (6 mm outer diameter) filled with Tenax TA 60/80 (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany). Air from the cylinder was sent to the glass bottle containing the odor source plant sample, and then to the Tenax tube at a flow rate of 100 mL/min. Sampling of the headspace volatiles was conducted for 1 h and volatile collection was repeated 5–9 times for each treatment.

The collected volatile compounds were analyzed using a gas chromatograph-mass spectrometer (GC-MS) (GC: Agilent Technologies, Inc., Santa Clara, CA, USA; 6890 with HP-5MS capillary column: 30 m long, 0.25 mm I.D., and 0.25 μm film thickness; MS: Agilent Technologies, Inc., 5973 mass selective detector, 70 eV) equipped with a separate desorption system, a cooled injection system, and a cold trap system (Gerstel GmbH & Co. KG). The headspace volatiles were tentatively identified by comparing their mass spectra with those from the Wiley databases (Wiley7N and

Wiley275). The mass spectra and retention times of detected volatile compounds were further compared with those of standard compounds [β -myrcene, (*Z*)-3-hexenyl acetate, linalool, methyl salicylate, indole, geranyl acetone, and (*E*)- β -farnesene; Wako Pure Chemical Industries, Ltd, Osaka, Japan, α -copaene; Fluka Chemie GmbH, Buchs, Germany, *cis*-jasmones; Tokyo Chemical Industries, Tokyo, Japan]. (*E*)- β -Ocimene, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), and (*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) were obtained from Dr. W. Boland. Compounds for which no standards were available were regarded as tentatively identified when more than a 90% of their spectra matched those of the databases.

Statistics. Comparisons were made between the following four pairs: (1) inexperienced females to nonhost-infested plants versus inexperienced females to host-infested plants, (2) inexperienced females to nonhost-infested plants versus experienced females to nonhost-infested plants, (3) experienced females to nonhost-infested plants versus experienced females to host-infested plants, and (4) inexperienced females to host-infested plants versus experienced females to host-infested plants. The effects of the treatment (combination of female experience and infested plants) on the ratios of taking-off and landing behavior of *C. kariyai* in the wind tunnel were analyzed by generalized linear mixed models (GLMMs) with a binomial distribution and logit-link using the function `glmer` in the package `lme4` version 1.17 (Bates et al. 2015) in R version 3.3.3 (R Core Team, 2017). The effects of the treatment on the duration of time from the taking-off to landing and that of host-searching behavior were analyzed using GLMMs with a normal distribution and identity-link using `lmer` in the package `lme4`. All duration data were Box-Cox transformed using JMP version 11.2.1 (SAS Institute, 2013) before the GLMMs. The replication was a random effect in all models. Significance was evaluated with the likelihood ratio test using the function `anova` and by comparing models with or without the effect of the treatment. Pairwise comparisons between nonhost-infested plants and host-infested plants, and between inexperienced females and experienced females were performed with Holm's sequentially rejective Bonferroni test (Holm, 1979) to adjust for the effects of multiple comparisons. We conducted a Wilcoxon rank sum test using the function `wilcox.test` in R to determine if there were differences in the relative amounts of volatile compounds emitted from maize leaves infested with host (CAW) and nonhost (TCW) larvae for *C. kariyai*.

RESULTS

Behavioral Responses of C. kariyai to Host-, and Nonhost-infested Maize Plants. Step-1: the relative ratio of taking-off (Fig. 1A). The ratio (%) that the inexperienced wasps showed toward nonhost-infested plants and that toward host-infested plants were not significantly different (the lowercase letters on the first versus the second bar) (GLMMs; $P = 0.8178$, $\alpha = 0.05$). The ratio that the experienced wasps showed toward nonhost-infested plants and that toward host-infested plants were significantly different (the lowercase letters on the third vs the fourth bar) (GLMMs; $P = 0.0127$, $\alpha = 0.0167$). Experience decreased the ratio toward nonhost-infested plants (the uppercase letters on the first versus the third bar) (GLMMs; $P = 0.0051$, $\alpha = 0.0125$) but not toward host-infested plants (the uppercase letters on the second versus the fourth bar) (GLMMs; $P = 0.7522$, $\alpha = 0.025$) (Fig. 1A and Table 1A).

Step-2: the duration from taking-off to landing (Fig. 1B). The flight duration of inexperienced wasps in experiments with nonhost-infested plants was not significantly shorter than that of experiments with host-infested plants (the lowercase letters on the first versus the second bar) (GLMMs; $P = 0.0679$, $\alpha = 0.0167$). The flight duration of experienced wasps was not significantly different in experiments with nonhost-infested and host-infested plants (the lowercase letters on the third versus the fourth bar) (GLMMs; $P = 0.3337$, $\alpha = 0.05$). Experience increased the duration significantly in experiments with nonhost-infested plants (the uppercase letters on the first versus the third bar) (GLMMs; $P = 0.0033$, $\alpha = 0.0125$), but not in those with host-infested plants (the uppercase letters on the second versus the fourth bar) (GLMMs; $P = 0.2861$, $\alpha = 0.025$). (Fig. 1B and Table 1B).

Step-3: the relative ratio of landing (Fig. 1C). The ratios (%) of landing that the inexperienced wasps showed toward nonhost-infested and host-infested plants were not significantly different (the lowercase letters on the first versus the second bar) (GLMMs; $P = 0.2852$, $\alpha = 0.025$). The ratios that the experienced wasps showed toward nonhost-infested and host-infested plants were significantly different (the lowercase letters on the third versus the fourth bar) (GLMMs; $P = 0.0011$, $\alpha = 0.0125$). Experience significantly decreased the ratios of landing toward nonhost-infested plants (the uppercase letters on the first versus the third bar) (GLMMs; $P = 0.0123$, $\alpha = 0.0167$) but

not toward host-infested plants (the uppercase letters on the second versus the fourth bar) (GLMMs; $P = 0.7025$, $\alpha = 0.05$) (Fig. 1C and Table 1C).

Step-4: the duration of searching on maize plants. The duration that the inexperienced wasps spent searching on nonhost-infested plants was significantly shorter than that on host infested plants (the lowercase letters on the first versus the second bar) (GLMMs; $P = 0.0075$, $\alpha = 0.025$). The experienced wasps spent significantly different durations searching on nonhost-infested and on host-infested plants (the lowercase letters on the third versus the fourth bar) (GLMMs; $P = 0.0024$, $\alpha = 0.0125$). Experience significantly decreased the searching durations on both nonhost-infested plants (the upper cases on the first versus the third bar) (GLMMs; $P = 0.0020$, $\alpha = 0.05$) and on host-infested plants (the upper cases on the second versus the fourth bar) (GLMMs; $P = 0.0062$, $\alpha = 0.0167$) (Fig. 1D and Table 1D).

Chemical Analyses. A total of 22 volatile compounds were detected in the headspace of maize plants infested with either host larvae or nonhost larvae (Table 2). Among them, the amounts of (*Z*)-3-hexenyl acetate ($P = 0.0496$: Wilcoxon rank sum test) and α -copaene ($P = 0.0450$: Wilcoxon rank sum test) were significantly higher in the nonhost-HIPVs than in host-HIPVs. We detected 10 compounds in the headspace volatiles from three detached maize leaves infested with 20 fourth-stadium nonhost larvae during sampling for 1 h (Table 2). Among them, (*Z*)-3-hexenyl acetate was the major compound, while α -copaene was not detected.

DISCUSSION

Are C. kariyai females attracted to HIPVs emitted from maize plants infested with nonhosts (TCWs)?

We showed that inexperienced *C. kariyai* females were attracted to nonhost (TCW)-HIPVs under the single-target experimental conditions, which indicated that the wasps would find nonhost-infested plants and consequently may show oviposition behavior towards nonhosts in field conditions. Although not significantly different (GLMMs; $P = 0.0679$, $\alpha = 0.0167$), the inexperienced wasps exhibited less flight time in Step-2 (the duration from taking-off to landing) to nonhost-infested plants than to host-infested plants (Fig. 1.B). This suggests that HIPVs from nonhost-infested plants are more attractive to *C. kariyai* than those from host-infested plants.

No qualitative differences were detected between the HIPVs from plants infested with hosts and nonhosts for 19 h. However, quantitative differences between the HIPVs from plants infested with hosts and nonhosts were detected, whereby there were significantly higher amounts of (Z)-3-hexenyl acetate and α -copaene in the nonhost-HIPVs than in the host-HIPVs. The shorter duration in Step-2 by inexperienced wasps to HIPVs from nonhost-infested plants than to those from host-infested plants may have been due to the higher amounts of (Z)-3-hexenyl acetate and/or α -copaene in nonhost-HIPVs. In a Y-tube olfactometer, inexperienced *C. kariyai* females are attracted to (Z)-3-hexenol but not to (Z)-3-hexenyl acetate (Takabayashi et al. 1991). It is noteworthy that the mode of the olfactory response of an insect in a Y-tube olfactometer and in a wind tunnel differs. Further studies are needed to assess if and how (Z)-3-hexenyl acetate and/or α -copaene are involved in the attraction of the inexperienced *C. kariyai*.

How does the experience of oviposition in nonhosts affect subsequent olfactory responses of C. kariyai females to host- and nonhost-HIPVs, and their antennal host searching behavior on host- and nonhost-infested plants?

We found that the negative experience (oviposition behavior to nonhosts) on a freshly nonhost-infested leaf negatively affected their subsequent olfactory response intensities to nonhost-HIPVs, but not to host-HIPVs in Step-1 to Step-3 (Fig. 1ABC). We hypothesized that the wasps associated the negative experience with the freshly produced nonhost-infested leaf volatiles, and exhibited reduced responses only to the nonhost-HIPVs. In the headspace of leaves infested with nonhost larvae for 1 h, (Z)-3-hexenyl acetate was the major compound while most of the other HIPVs (i.e., volatile terpenoids) from host- and nonhost-infested plants, including α -copaene, were not detected (Table 2). This absence can be explained by the fact that most of the herbivory-induced volatile terpenoids are synthesized *de novo* in plants and are emitted ~1 day after herbivory (Arimura et al. 2008). In contrast, (Z)-3-hexenyl acetate is one of the green leaf volatiles that are emitted immediately after mechanical wounding or herbivory (Matsui 2006).

The relative amount of (Z)-3-hexenyl acetate in nonhost-HIPVs was significantly higher than in host-HIPVs, and (Z)-3-hexenyl acetate was also found in volatiles from freshly nonhost-infested leaves. Collectively, the chemical and biological

data supported our hypotheses: *C. kariyai* would have associated the negative experience with the (Z)-3-hexenyl acetate emitted from freshly nonhost-infested leaves, and this experience ultimately reduced their responses to the nonhost-HIPVs, in which the higher amount of (Z)-3-hexenyl acetate was involved. The question of why *C. kariyai* did not show negative responses to host-HIPVs, even though the smaller amount of (Z)-3-hexenyl acetate was also in the host-HIPVs, still remains unanswered.

In Step-4, the longer that the *C. kariyai* females search on a plant, the higher the possibility that they would find a host or nonhost. Interestingly, after negative experience, the wasps spent significantly less time searching on both host- and nonhost-infested plants (Fig.1 D). On host-infested plants, *C. kariyai* females were seen to exhibit antennal contact with the host-infested edges of a leaf and host feces, and this caused the longer duration of time on the host-infested plants than on uninfested plants (Sato et al. 1983; Takabayashi et al. 1985). One of the factors that elicit the antennal searching behavior of *C. kariyai* females on host-infested edges and host feces is a series of 2,5-dialkyltetrahydrofuran homologs (mainly from C27 to C35) (Takabayashi and Takahashi 1986ab). During negative experiences on nonhost-infested leaves, *C. kariyai* may have perceived nonhost-derived cues that elicited the antennal searching of the wasps on the leaves. It still remains to be elucidated whether *C. kariyai* females associate negative experiences with such cues and, therefore, explain the reduced searching duration in Step-4.

This study showed, for the first time, that the oviposition behavior to nonhosts (negative experience) on a nonhost-infested leaf negatively affected the subsequent responses to nonhost-HIPVs. To date, most studies have focused on positive experience (e.g., oviposition success and host-finding cues) with host-HIPVs (e.g., Costa et al. 2010; Fukushima et al. 2002). A comparative study on the effects of positive and negative experiences with synthetic host-HIPVs in attracting *Cotesia vestalis*, a specialist parasitoid of diamondback moth larvae, has been reported (Uefune et al. 2013). It is adaptive for wasps to become choosier in their responses to detected host-HIPVs after a negative experience. However, wasps spent less time not only on nonhost-infested plants but also on host-infested plants after a negative oviposition experience, and the reduction of time spent on host-infested plants is not considered to adaptive for the wasps. Our study indicated that the density (i.e., possible encounters) of

351 nonhost species as well as that of host species should be considered when assessing the
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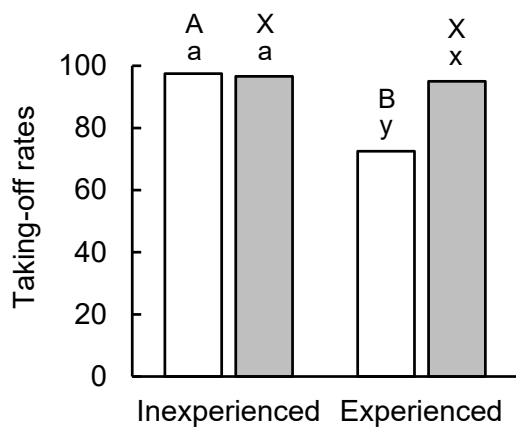
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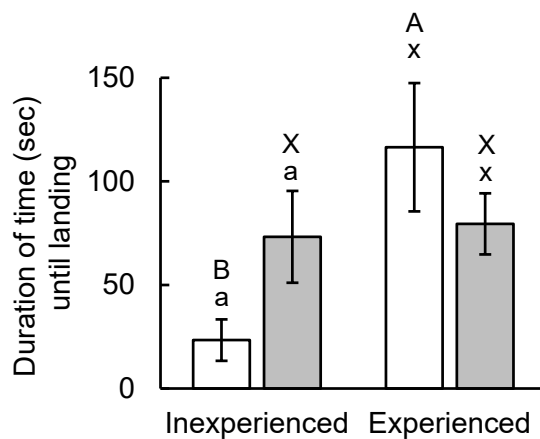
Figure legend

Fig. 1 The flight responses to infested plants (Step-1 to Step-3) and the host searching response on infested plants (Step-4) of *Cotesia kariyai* in the wind tunnel. The different lowercase letters for the data of the same wasp condition (either inexperienced or experienced) indicate significant differences by GLMM based on each Holm-adjusted significance level between nonhost-infested and host-infested plants. The different uppercase letters for the data of the same plant condition [either nonhost-infested (white bars) or host-infested (gray bars)] indicate significant differences by GLMM based on each Holm-adjusted significance level between inexperienced and experienced females. Thirty (the response of inexperienced female to host-infested plants) and 40 individuals (others) were tested.

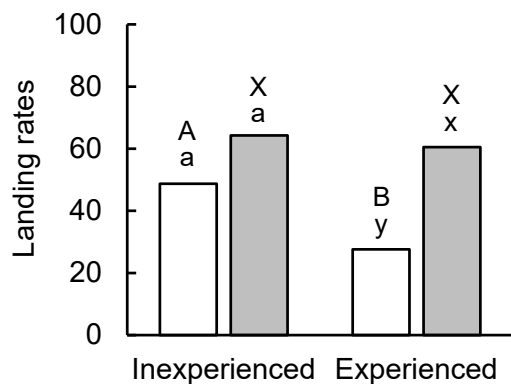
(A) Step-1: the relative ratio (%) of taking-off



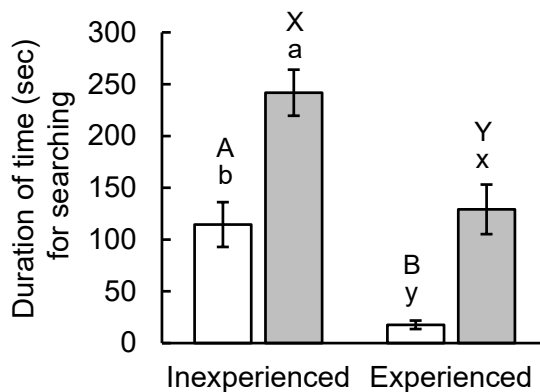
(B) Step-2: the durations of time from the taking-off to landing



(C) Step-3: the relative ratio (%) of landing



(D) Step-4: : the duration of searching on plants



Nonhost-infested plant

Host-infested plant

Table 1 Summary table showing degrees of freedom (df), chi-squared values (χ^2), P -values (P), Holm-adjusted significance level (α) after generalized linear mixed models (GLMMs) for the differences of each step in treatment in combination with experience and infested plant.

(A) Step-1: the relative ratio (%) of taking-off

Treatment	df	χ^2	P	α
IF to NIP vs IF to HIP	1	0.053	0.8178	0.05
IF to NIP vs EF to NIP	1	7.856	0.0051 *	0.0125
EF to NIP vs EF to HIP	1	6.1888	0.0127 *	0.0167
IF to HIP vs EF to HIP	1	0.0997	0.7522	0.025

(B) Step-2: the durations of time from the taking-off to landing

Treatment	df	χ^2	P	α
IF to NIP vs IF to HIP	1	3.3339	0.0679	0.0167
IF to NIP vs EF to NIP	1	8.6596	0.0033 *	0.0125
EF to NIP vs EF to HIP	1	0.9346	0.3337	0.05
IF to HIP vs EF to HIP	1	1.1377	0.2861	0.025

(C) Step-3: the relative ratio (%) of landing

Treatment	df	χ^2	P	α
IF to NIP vs IF to HIP	1	1.1422	0.2852	0.025
IF to NIP vs EF to NIP	1	6.2631	0.0123 *	0.0167
EF to NIP vs EF to HIP	1	10.624	0.0011 *	0.0125
IF to HIP vs EF to HIP	1	0.1459	0.7025	0.05

(D) Step-4: : the duration of searching on plants

Treatment	df	χ^2	P	α
IF to NIP vs IF to HIP	1	7.1515	0.0075 *	0.025
IF to NIP vs EF to NIP	1	9.5365	0.0020 *	0.05
EF to NIP vs EF to HIP	1	9.2325	0.0024 *	0.0125
IF to HIP vs EF to HIP	1	7.5053	0.0062 *	0.0167

IF: Inexperienced females, EF: experienced females, NIP: nonhost infested plants. HIP: host infested plants. The asterisk indicates significant P -value based on Holm-adjusted significance level.

Table2. Volatile compounds emitted from maize leaves infested with host (*Mythimna separate*) and nonhost (*Spodoptera litura*) caterpillars of *Cotesia kariyai* for different durations of time (1 h and 19 h)

Compound	Relative amounts / gFW							
	1 h Nonhost				19 h			
	Median (1st–3rd quartiles)				Median (1st–3rd quartiles)			
					Nonhost		Host	
β-myrcene	ND				0.009	(0.000 - 0.027)	0.006	(0.000 - 0.019)
(Z)-3-hexenyl acetate	0.128 (0.078 - 0.338)				0.276	(0.172 - 0.994)	0.062 *	(0.017 - 0.253)
(E)-β-ocimene	ND				0.048	(0.003 - 0.080)	0.030	(0.008 - 0.118)
linalool	0.044 (0.031 - 0.071)				0.468	(0.103 - 0.907)	0.450	(0.149 - 2.470)
DMNT	0.020 (0.017 - 0.084)				0.394	(0.233 - 1.401)	0.645	(0.206 - 1.763)
methyl salicylate	0.014 (0.004 - 0.030)				0.000	(0.000 - 0.011)	0.000	(0.000 - 0.010)
indole	ND				0.553	(0.262 - 2.749)	1.337	(0.128 - 4.509)
unidentified compound	0.044 (0.037 - 0.069)				0.121	(0.092 - 0.150)	0.079	(0.062 - 0.134)
ylangene†	0.037 (0.028 - 0.059)				0.113	(0.090 - 0.141)	0.071	(0.043 - 0.117)
α-copaene	0.015 (0.011 - 0.021)				0.019	(0.007 - 0.034)	0.003 *	(0.000 - 0.010)
cis-jasmone	ND				0.000	(0.000 - 0.018)	0.013	(0.000 - 0.022)
unidentified sesquiterpene 1	ND				0.046	(0.026 - 0.053)	0.026	(0.020 - 0.049)
unidentified sesquiterpene 2	ND				0.166	(0.073 - 0.264)	0.081	(0.036 - 0.274)
β-sesquiphellandrene†	ND				0.000	(0.000 - 0.000)	0.000	(0.000 - 0.020)
geranyl acetone	0.005 (0.000 - 0.008)				0.025	(0.000 - 0.163)	0.039	(0.012 - 0.083)
(E)-β-farnesene	ND				0.748	(0.314 - 0.997)	0.292	(0.119 - 1.056)
unidentified sesquiterpene 3	ND				0.015	(0.000 - 0.044)	0.015	(0.000 - 0.024)
unidentified sesquiterpene 4	ND				0.000	(0.000 - 0.024)	0.008	(0.000 - 0.020)
α-amorphene†	0.021 (0.014 - 0.031)				0.062	(0.050 - 0.095)	0.052	(0.027 - 0.103)
δ-cadinene†	0.015 (0.011 - 0.027)				0.053	(0.051 - 0.095)	0.033	(0.026 - 0.070)
unidentified sesquiterpene 5	ND				0.022	(0.000 - 0.026)	0.017	(0.011 - 0.026)
TMTT	ND				0.109	(0.061 - 0.197)	0.037	(0.024 - 0.159)

ND, not detected.

Data represent the mean \pm SE of 5-9 replications. Values with asterisks are significantly different ($P < 0.05$, Wilcoxon rank sum test) between nonhost-infested plant and host-infested plant. Compounds with † are tentatively identified.

DMNT; (*E*)-4,8-dimethyl-1,3,7-nonatriene , TMTT; (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene