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ORIGINAL ARTICLE

Colletes hederae bees are equally attracted by visual and olfactory cues of inconspicuous *Hedera helix* flowers

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Abstract

Wild bees are heavily declining worldwide except for a few species, such as *Colletes hederae*, which is spreading in its distribution throughout Europe. *Colletes hederae* mainly forages on ivy (*Hedera helix*) which is widespread in Europe and the plants' availability is thought to contribute to the successful spread of *C. hederae*. A rapid location of the plants using visual and/or olfactory floral cues would allow the bee to efficiently forage. Beside bee visitors, the flowers attract a high variety of other insects, such as *Vespula* wasps that were recently investigated regarding their floral-cue preferences. The aim of this study was to investigate the communication between *C. hederae* and its *H. helix* host flowers, and to compare the results with that previously obtained with *V. germanica* wasps. We identified headspace compounds detectable by the bees using gas chromatography coupled to electroantennography (GC-EAD) and performed behavioral experiments to both compare the attractiveness of visual and olfactory floral cues and to determine the attractiveness of a synthetic mixture composed of physiologically active compounds. In the GC-EAD analyses, bees responded to 15 flower-specific compounds of various chemical classes, of which 4-oxoisophorone, (*E*)-linalool-oxide furanoid, and acetophenone were the most abundant in the floral scent. In the bioassays, visual and olfactory flower cues were equally attractive for bees, but a combination of both cues was needed to elicit not only approach responses but also landings. A synthetic mixture of the EAD-active compounds was attractive to the bees, but to a lesser extent than the natural scent of *H. helix* flowers. The bees' integrations of different floral-cue modalities in its search image and its strong antennal responses elicited by various floral scent compounds make *C. hederae* highly effective in finding its host flowers. In comparison to *V. germanica* wasps, the bees relied stronger on visual cues than the wasps do, but both species showed the highest attraction when presented with a combination of the cues.

Keywords Wild bees · Floral scent · Foraging behavior · Flower preferences · Floral signals · Host-plant finding

Introduction

Wild bees are a diverse group of insects with more than 20,000 species worldwide (Michener 2007). They are highly effective pollinators of a huge variety of plant species (Klein et al. 2007; Ollerton et al. 2011; Garibaldi et al. 2013). For foraging, bees often restrict their visits to a subset of available plant species (Kuppler et al. 2023), mainly due to nectar and pollen properties, flower morphology, and plant abundance (van der Kooi et al. 2021). To locate their host plants, bees use mainly visual and olfactory floral cues (Burger et al. 2021; Rachersberger et al. 2019). Host-specific volatiles are, thereby, particularly important for oligolectic (pollen-specialist) bees to recognize their specific pollen hosts (Burger et al. 2011; Schäffler et al. 2015). Host plants of oligolectic bees are often visited by various pollinating species but comparable studies about their floral-cue preferences are rare (but see, e.g., Milet-Pinheiro et al. 2016).

The common ivy, *Hedera helix,* hosts a wide range of floral visitors (Ollerton et al. 2007; Jacobs et al. 2010), but the relative importance of floral signals of this generalist plant has been studied only for *Vespula* wasps (Lukas et al. 2020). *Hedera helix* flowers are visually inconspicuous, as they

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appear within the dense leaf vegetation and have similar coloring to the leaves. The scent of the flowers is, in contrast, highly distinct from the leaves and emitted in high amounts (Lukas et al. 2020). *Vespula germanica* wasps use mainly scent cues to find the inflorescences (Lukas et al. 2020). However, different floral visitors can rely to differing extents on the distinct sensory modalities to find flowers (Balkenius et al. 2006). *Hedera helix* flowers are also frequently visited by bees, such as *Colletes hederae* Schmidt & Westrich 1993 (Colletidae) (Westrich 2008). This bee species relies mainly on *H. helix* flowers as pollen and nectar resources but visits also other plant species, e.g., of the plant family Asteraceae (Westrich 2008). However, the floral cues that *C. hederae* uses to find its host flowers are unknown until now.

Colletes hederae has recently experienced increasing populations and has spread widely in Europe (Bischoff et al. 2005; Bogusch et al. 2021; Burger 2008; Hopfenmüller 2014a; Saure et al. 2019). In contrast, the majority of wild bees have declined worldwide in recent decades (Zattara and Aizen 2021). Habitat loss has been shown to have the greatest impact on bee populations due to the loss of floral resources and nesting sites (Brown and Paxton 2009; Scheper et al. 2014). However, *H. helix,* the main host plant of *C. hederae,* is not dramatically decreasing compared to the majority of native plant species in Germany (Eichenberg et al. 2021). Sufficient feeding options together with available nesting sites (Westrich 2008), favorable climatic conditions (Bischoff et al. 2005), and building of large bee populations in nesting aggregations (Schmid-Egger 1997) are thought to explain the intense spread of *C. hederae* in Europe. Further, a rapid location of the plants using visual and/or olfactory floral cues would allow the bee to efficiently forage and to provision a high number of brood cells within limited time.

The aim of this study was to identify the floral cues of *H. helix* responsible for attracting *C. hederae* bees and to compare the cues used by previously studied *V. germanica* wasps (Lukas et al. 2020). We performed electrophysiological and chemical analyses, as well as behavioral experiments with flowering stems and synthetic floral scent analogs to answer the following specific questions: (1) What is the relative importance of *H. helix* visual and olfactory floral cues for *C. hederae*? (2) Which volatiles of the floral scent bouquet of *H. helix* elicit antennal responses in *C. hederae?* (3) Is a synthetic mixture of electrophysiologically active components as attractive to *C. hederae* as the entire assemblage of olfactory cues from flowering stems of *H. helix*?

Material and methods

Study organisms and study sites

Perennial *Hedera helix* (Araliaceae) is widely distributed in Europe and flowers from August to November (Metcalfe 2005). For this study, flowers were sampled at Ulm (Germany), Rosenheim (Germany), and Salzburg (Austria) in 2018 and 2019.

Fig. 1 *Colletes hederae* female visiting a *Hedera helix* inflorescence

The bee species *Colletes hederae* (Fig. 1) is distributed in southern and central Europe. The bees are active between mid-September and October (Amiet and Krebs 2014; Westrich 2018) and build their nests in the ground, often in large aggregations (several hundred individuals). For this study, female *C. hederae* bees were collected during foraging flights on *H. helix* flowers at several dates in Ulm and once in Meersburg in September 2019, Germany. The collected bees were released to a flight cage, located in the Botanical Garden of Ulm, for behavioral experiments or they were stored in the dark in a fridge (ca. 4 °C) for max. 72 h before using them for electrophysiological experiments. The flight cage (2 m × 3 m × 3 m) was located in the Botanical Garden of Ulm; inside the cage, the bees were offered *H. helix* flowering stems and sugar water.

Floral scent samples

Scent samples for this study were used from the study of Lukas et al. (2020): Sample remnants were used for electrophysiological experiments and identification of physiologically active compounds, and data of further samples were re-analyzed for compound quantifications. For electrophysiological experiments, additional scent samples were collected following the same methods as Lukas et al. (2020).

Two different sample types were collected/analyzed for different purposes: Solvent samples were used for electrophysiological analyses and compound identification. Thermal desorption (TD) samples were used for compound quantification. The latter ones were collected from various plant individuals to describe the variance of a wider range of individuals from different study sites.

The method used by Lukas et al. (2020) to obtain scent samples by dynamic headspace (Dötterl and Jürgens 2005) was as follows: *Hedera helix* inflorescences or vegetative plant parts (one leaf stalk with ten leaves each) were

enclosed in polyethylene oven bags (Toppits®, Melitta, Germany) and scent was trapped using adsorbent tubes filled with Tenax TA (mesh 60 80) and Carbotrap B (mesh 20 40; both Supelco, Bellefonte, PA, USA). Scent samples collected from empty oven bags were used as negative controls. Solvent samples were obtained from adsorbent tubes (Duran glass capillaries; outer diameter 6.0 mm; inner diameter 4.0 mm; length 80 mm; Paul Stollwerk Glasbläserei, Emmerting, Germany) filled with 10 mg of each adsorbent and eluted with 80 µl acetone (GC/HPLC Grade, Rotisolv, Carl Roth GmbH + Co KG, Karlsruhe) after 5 h sampling time. For TD samples, smaller adsorbent tubes (quartz glass capillaries; outer diameter 2.5 mm; inner diameter 1.9 mm; length 25 mm; Hilgenberg GmbH, Malsfeld, Germany) were used and filled with 1.5 mg of each adsorbent. Volatiles were trapped for only 10 min.

Electrophysiological experiments

The physiological activity of *H. helix* volatiles on the antennae of *C. hederae* was analyzed using gas chromatography coupled to electroantennographic detection (GC-EAD) following Lukas et al. (2020). We used four headspace solvent samples collected from *H. helix* inflorescences for the GC-EAD experiments. Two thereof were from a previous study (Lukas et al. 2020).

The GC-EAD set-up consisted of a gas chromatograph (Agilent 7890 A Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and an electroantennographic detection system (EAD). The EAD system was equipped with a transfer line and a 2-channel USB acquisition controller (IDAC-2; Syntech, Kirchzarten, Germany). The GC runs were performed with the following parameters: ZB-5 fused silica column (30 m-long, inner diameter 0.32 mm, film thickness 0.25 µm, Phenomenex); carrier gas hydrogen 3 mL min^{-1}; 1 µl sample injection in splitless mode at 250°C; oven temperature 40°C (0.5 min), increased by 10 °C min^{-1} to 220 °C (2 min). The column was split (four-way microflow splitter, Gerstel, Mühlheim, Germany) into two deactivated capillaries of which one led to the FID (2 m × 0.15 µm inner diameter), one to the EAD (1 m × 0.2 µm inner diameter), and a make-up gas (N_2; flow: 25 mL min^{-1}) was introduced through the fourth arm of the splitter. The outlet of the EAD was placed in a cleaned and humidified airflow (tube inner diameter: 7.5 mm) directed over the bee antenna (Heiduk et al. 2015). Antennae from *C. hederae* females were cut off at the base and tip and positioned between two capillaries with extended tip filled with Ringer solution (8.0 g L^{-1} NaCl, 0.4 g L^{-1} KCl, 0.4 g L^{-1} $CaCl_2$) and connected via silver wires to close the electrical circuit. Responses of 12 bees were analyzed. Volatiles to which five or more bee individuals responded were considered as EAD-active.

Compound identification and quantification

Electrophysiologically active peaks were identified by injecting the solvent scent samples into a GC–MS system of Shimadzu (QP2010 Ultra, helium as carrier gas, ZB-5 fused silica column, 30 m-long, inner diameter 0.32 mm, film thickness 0.25 µm, Phenomenex, Aschaffenburg, Germany; electron ionization: 70 eV; *m/z* range: 35–350) as described by Lukas et al. (2020). All compounds identified as EAD-active based on solvent samples were considered for the analysis of TD samples. The TD samples run by Lukas et al (2020) on a GC–MS system were re-analyzed to calculate mean relative amounts (± standard error) of EAD-active floral compounds. The samples were analyzed with an automatic thermal desorption system (model TD-20, Shimadzu, Japan) connected to a gas chromatograph coupled to mass spectrometry (GC–MS, model QP2010 Ultra El, Shimadzu, Japan) equipped with a ZB-5 fused silica column (5% phenyl polysiloxane; 60 m-long, inner diameter 0.25 mm, film thickness 0.25 mm, Phenomenex, Aschaffenburg, Germany). To identify compounds that were emitted by inflorescences and not only by vegetative parts, the GC–MS runs were compared between inflorescence ($N = 18$), vegetative ($N = 15$), and blank control samples ($N = 9$). Compounds found in blank control samples were considered as contaminants and excluded from the analyses. Volatiles were classified as floral if they were only found in inflorescence samples or if they occurred in vegetative samples but at higher amounts in inflorescence samples.

Obtained data were processed using the GCMSsolution package, Version 4.41 (Shimadzu Corporation). Compounds were tentatively identified using both the mass spectral libraries Adams (2007), W9N11, NIST 11, FFNSC 2, and ESSENTIAL OILS (available in MassFinder 3) and literature data on retention indices based on n-alkane series (van den Dool and Kratz 1963). The identity of the compounds was confirmed using synthetic standard compounds, if they were available in the reference collection of the Plant Ecology Laboratory of the Paris Lodron University of Salzburg.

Behavioral experiments

Bioassays were performed with foraging-experienced *C. hederae* females present in the flight cage (2 m × 3 m × 3 m). Inside the cage, the bees were offered *H. helix* flowering stems and sugar water. One hour before the experiments started, the food sources were removed. The number of bees in the cage during each experiment varied between approx. 20 and 40 individuals.

Experimental set-up

The attractiveness of visual and olfactory inflorescence signals of *H. helix* was investigated with two-choice cylinder (29 cm height, 10 cm diameter) experiments following Burger et al. (2010), that also graphically illustrated the used cylinders. To examine visual cues, transparent cylinders made of acrylic UV-transmitting glass without holes, for olfactory cues, black cylinders with small holes that allowed the diffusion of scents but blocking visual cues, and for the combination of inflorescence olfactory and visual cues transparent cylinders with holes were used. In detail, we tested (a) inflorescence visual cues against olfactory cues, (b) the combination of inflorescence olfactory and visual cues against olfactory cues, (c) the synthetic inflorescence scent sample (see below) against a solvent control (mineral oil: Sigma-Aldrich, BioUltra, Darmstadt, Germany), and (d) the synthetic inflorescence scent sample plus visual inflorescence cues against a solvent control plus visual inflorescence cues and (e) the synthetic inflorescence scent sample against inflorescence olfactory cues. For experiments with inflorescences, five flowering twigs (picked 3–6 h before the experiments and kept fresh in vases with water) were placed in the cylinders; volatiles were pumped out of the cylinders with holes using a membrane pump (flow: 1 L min^{-1}; G12/01 EB, Rietschle Thomas Inc., Puchheim, Germany). In the experiment testing olfactory versus visual cues, a transparent cylinder, instead of the black cylinder, with holes was used for the olfactory set-up to avoid a bias caused by differently colored cylinders: flowering twigs were placed in an external glass container connected to the experimental cylinder via silicon tubes, and the external container was covered with aluminum foil. Synthetic scent was investigated using the black cylinders with holes: the synthetic solution was applied on rectangular sponge strips (35 × 7.5 × 1.5 mm, length × width × thickness; Kettenbach GmbH & Co. KG, Eschenburg, Germany) and placed on aluminum foil to avoid contamination of the cylinder; the synthetic solution and the mineral oil were applied twice during the experiment, at the beginning and midway. To investigate the synthetic scent in combination with visual cues, the same black cylinder with holes was used and a transparent cylinder (8 cm height, 10 cm diameter) containing inflorescences was placed on top.

Experimental procedure

The two choices were offered 1 m apart from each other on a wooden table. Each experiment lasted 1 h, during which the position of the cylinders was exchanged after 30 min. Only bees that directly approached (10 cm minimum distance to the cylinder) or landed on the cylinders were recorded. We caught the responding bees and stored them in small glass containers in a cooling box until the experiment was finished. The behavioral experiments testing the synthetic mixture against a negative control were conducted twice on different days because of low number of responses and the data of the trials were pooled. Experiments were performed on warm and sunny days between 10 am and 3 pm. The data were analyzed using exact binomial tests. A Fisher's exact test was used to compare approaches and landings to the synthetic mixture in combination with and without visual cues.

Synthetic mixture

The synthetic solution used in the experiments resembled quantitatively and qualitatively the inflorescence scent of ivy according to Table 1. Synthetic analogs of the EAD-active floral compounds were used (Table 2; dihydrooxoisophorone was not available). The compounds were added as a pure substance or diluted in mineral oil (Sigma-Aldrich, BioUltra, Darmstadt, Germany) to the stock solution of the synthetic mixture according to Table 2. All compounds except for β-ocimene, linalool-oxide pyranoid (BOC Science), and acetophenone (Fluka) were obtained from Sigma-Aldrich, and all compounds except for phenylacetaldehyde (90%) had a purity of at least 95%. The solution had a total volume of 273 µl, whereof 27.3 µl were taken and mixed with 972.7 µl mineral oil. In a next step, the solution was diluted 1:10 in mineral oil. An amount of 100 µl of the final solution was added to the sponge strips. GC–MS runs of headspace samples collected from the synthetic mixture were compared to inflorescence samples to determine the released amount of the compounds.

Results

GC-EAD-active compounds

The inflorescence scent of *H. helix* contained 32 compounds that elicited antennal responses in *C. hederae* bees, of which 21 were flower-specific (Table 1, Fig. 2). Among the EAD-active compounds were 17 terpenoids, seven aromatics, two nitrogen-containing, one aliphatic, and five unknown compounds (Table 1). The most abundant flower-specific compounds were 4-oxoisophorone (49.2 ± 5.4%, mean ± standard error of total amount of the EAD-active compounds), (*E*)-linalool-oxide furanoid (11.0 ± 3.5%), acetophenone (10.2 ± 2.4%), (*Z*)-linalool-oxide furanoid (5.8 ± 1.6%), and dihydrooxoisophorone (5.3 ± 0.8%). None of the other compounds exceeded a mean relative amount of 5%. The total amount of the physiologically active scent was 1498 ± 292 ng $umbel^{-1}$ h^{-1} (mean ± standard error)

Table 1 Total and relative amounts (mean ± standard error) of electrophysiologically active volatiles of *Hedera helix* inflorescences and vegetative parts eliciting responses from *Colletes hederae* antennae

No	Volatile compounds	RI	Inflorescences ($n = 18$)	Vegetative parts ($n = 15$)	Bee responses ($n = 12$)
	Total number of volatiles		18.0 ± 0.7	7.0 ± 0.5	
	Total amount of scent [ng h^{-1}][a]		1498 ± 291	88.1 ± 38.4	
	Aliphatic compounds[b]		**0.7 ± 0.6**	–	
1	2-Heptanol* (f)	900	0.7 ± 0.6	–	11
	Aromatic compounds[b]		**19.1 ± 1.3**	**14.4 ± 1.7**	
2	Benzaldehyde*	967	3.4 ± 0.6	12.7 ± 3.3	12
5	4-Methylanisole*[c]	1024	–	–	8
7	Phenylacetaldehyde* (f)	1052	0.6 ± 0.2	0.0 ± 0.0	12
8	1-Phenylethanol (f)	1065	0.1 ± 0.1	0.0 ± 0.0	7
9	Acetophenone* (f)	1073	10.2 ± 2.4	1.7 ± 0.6	12
12	Methyl benzoate* (f)	1101	3.9 ± 1.2	–	10
13	2-Phenylethanol* (f)	1122	0.7 ± 0.2	–	12
	Nitrogen-containing compounds[b]		**0.3 ± 0.1**	**–**	
20	1-Nitro-2-phenylethane* (f)	1308	0.0 ± 0.0	–	12
20	2-Aminoacetophenone* (f)	1312	0.3 ± 0.1	–	12
	Terpenoids[a]		**78.5 ± 2.8**	**85.6 ± 2.6**	
3	ß-Myrcene*	993	1.0 ± 0.4	15.4 ± 3.2	12
4	α-Phellandrene*	1010	1.0 ± 0.5	2.3 ± 0.8	7
6	ß-Phellandrene*	1037	1.3 ± 0.6	9.6 ± 2.3	10
6	(*Z*)-ß-Ocimene* (f)	1039	0.2 ± 0.1	0.4 ± 0.3	10
7	(*E*)-ß-Ocimene* (f)	1050	1.0 ± 0.3	0.6 ± 0.1	12
10	(*Z*)-Linalool oxide furanoid* (f)	1079	5.8 ± 1.6	–	11
11	(*E*)-Linalool oxide furanoid* (f)	1094	11.0 ± 3.5	–	12
12	Linalool* (f)	1100	0.5 ± 0.3		
14	4-Oxoisophorone* (f)	1150	49.2 ± 5.4	13.0 ± 4.0	12
15	Dihydrooxoisophorone (f)	1173	5.3 ± 0.8	1.1 ± 0.7	12
16	(*Z*)-Linalool oxide pyranoid* (f)	1176	1.3 ± 0.3	–	11
16	(*E*)-Linalool oxide pyranoid* (f)	1180	0.3 ± 0.1		11
21	δ-Elemene	1349	–	0.6 ± 0.5	7
22	Geranylacetone*	1457	0.7 ± 0.2	42.5 ± 6.7	11
22	(*E*)-ß-Farnesene*[c]	1461	–	–	11
23	Germacrene D*	1500	–	0.1 ± 0.1	10
24	(*E*,*E*)-α-Farnesene*[c]	1512	–	–	7
	Unknown[b]		**1.4 ± 0.1**	**–**	
17	*m/z:* 91, 43, 65, 119, 162, 39 (f)	1215	0.1 ± 0.1	–	11
18	*m/z:* 43, 60, 73, 57, 41, 55[d]	1256	–	–	8
19	*m/z:* 58, 43, 85, 57, 141, 69 (f)	1265	0.3 ± 0.1	–	11
25	*m/z:* 69, 41, 123, 138, 39 (f)	1609	0.2 ± 0.1	–	8
26	*m/z:* 69, 41, 123, 138, 39 (f)	1647	0.8 ± 0.2	–	9

Compounds are listed by compound class (bold names) according to Knudsen et al. (2006) and retention index (RI). The mass-to charge ratio *(m/z)* is given in decreasing order of abundance for unknown compounds. Numbers (No) correspond to numbered EAD-responses given in Fig. 2. Compound identification was verified through authentic standards, if available (*). Compounds were classified as floral (f) if they were found exclusively in inflorescence samples or in higher amounts in flower than in vegetative samples

[a]Absolute emissions refer to one umbel (8 umbellules) in inflorescence samples and stems with 10 leaves in vegetative samples

[b]In % of total amount

[c]EAD-active in solvent headspace samples but not found in thermal desorption samples

Table 2 Composition of the synthetic mixture tested in behavioral experiments

Compound name	Stock solutions of single compounds		Stock solution synthetic mixture (µl)
	Pure substance (µl)	Mineral oil (µl)	
2-Heptanol	37	963	15[d]
(*Z*)-ß-Ocimene[a]	148	852	15[d]
(*E*)-ß-Ocimene[a]			
Phenylacetaldehyde	203	797	35[d]
1-Phenylethanol	113	887	10[d]
Acetophenone			6
(*Z*)-Linalool oxide furanoid[b]			50
(*E*)-Linalool oxide furanoid[b]			
Linalool			7
Methyl benzoate			4
2-Phenylethanol			2
4-Oxoisophorone			120
(*Z*)-Linalool oxide pyranoid[c]			5
(*E*)-Linalool oxide pyranoid[c]			
2-Aminoacetophenone			4
Sum			273

A diluted aliquot of the stock solution was used for bioassays

[a,b,c]Compounds were only available in a mixture of isomers; [d]: amount added from stock solutions of single compounds

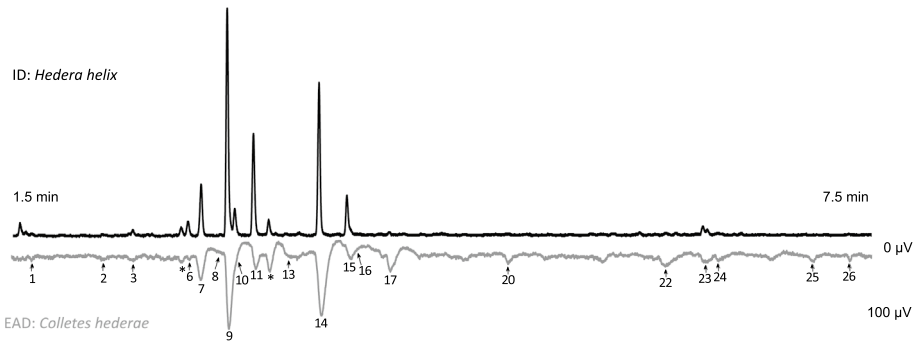

Fig. 2 Representative example of the electroantennographic responses of a *Colletes hederae* antenna (EAD: electroantennographic detection) to a floral scent sample of *Hedera helix* (FID: flame ionization detection). Numbered responses correspond to numbers given in Table 1 (*: response to contamination)

and 88 ± 38 ng leaf^{-1} h^{-1} in floral and vegetative samples, respectively.

Behavioral experiments

Decoupled visual and decoupled olfactory inflorescence cues of *H. helix* were equally attractive for *C. hederae* bees (Fig. 3). The synthetic solution alone (Fig. 3) or in combination with visual cues (Fig. 3) attracted significantly more bees than the negative controls. While the synthetic solution mainly induced the bees to approach, the combinational approach of synthetic scent and visual cues induced the bees mainly to land (Fisher's exact test, $P < 0.01$). The olfactory cues of *H. helix* inflorescences attracted significantly more *C. hederae* bees than the synthetic solution (Fig. 3).

Discussion

The behavioral experiments demonstrated that the bees responded most strongly when the experimental floral cues most closely resembled and matched *Hedera helix* flowers. Both decoupled visual and decoupled olfactory cues of *H. helix* were attractive for *Colletes hederae* bees, but only the combination of both cues efficiently induced landing behaviors. This suggests that the bees rely on a combination of these two cue modalities to recognize their specific foraging plant species. A synthetic mixture of *H. helix* compounds that elicited electrophysiological responses in the bee's antennae was behaviorally active, but less so than natural olfactory cues.

The finding that visual cues have the same attractiveness to *C. hederae* bees as the strong olfactory cues is

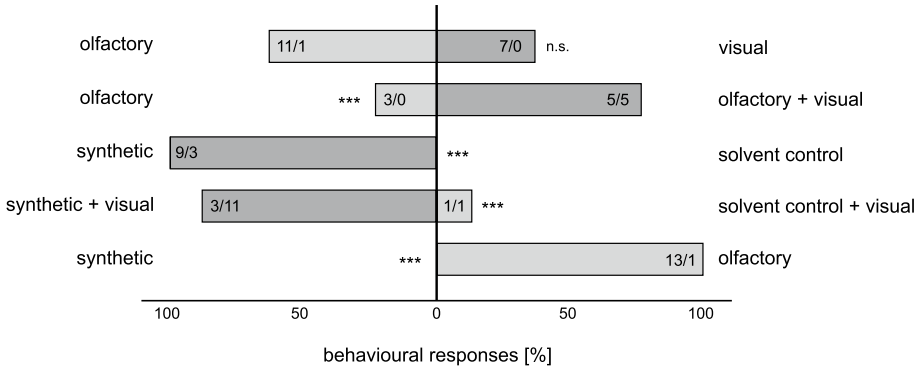

Fig. 3 Attractiveness of olfactory and visual cues of inflorescences of *Hedera helix* and of synthetic scent for *Colletes hederae* females in two-choice experiments. Numbers in the bars show the absolute number of bees that approached/landed (Fisher's exact test, *n.s.* > 0.05, ***: $p < 0.001$)

surprising, because the flowers form only a weak visual contrast for bees against the background vegetation (Lukas et al. 2020). The flowers are presented within a dense leaf vegetation and only the gynoecium in the center of the flowers shows a clear color contrast to the leaves (Lukas et al. 2020). The bees might have learned to discriminate between the subtle color differences during previous flower visits, because they were already foraging experienced when tested in behavioral experiments (Menzel 1985; Gumbert 2000; Dyer and Chittka 2004). Equal attractiveness between olfactory and visual cues is in accordance with some other studies (Milet-Pinheiro et al. 2012; Rachersberger et al. 2019), but often a strong color contrast explains a high attractiveness, which is not the case in this interaction. In combination with olfactory cues, floral visitors often use scent as a long-distance attractant and, close to flowers, orientate toward visual targets to actually land (Lunau 1992; Shuttleworth and Johnson 2009; du Plessis et al 2018). *Colletes hederae* bees also showed efficiently landing behaviors only when the combination of both cue modalities was presented. In comparison to *Vespula germanica* wasps (Lukas et al. 2020), *C. hedera* bees seem to rely more strongly on vision. Although the set of performed behavioral experiments differ between the two species and the results cannot be compared directly, *C. hederae* and *V. germanica* seem to differ in their choice behaviors when they are presented with decoupled floral cues. Whereas the visual cues had the same attractiveness as olfactory cues for *C. hederae*, which implies that decoupled visual cues were attractive to some extent, visual cues were not significantly more attractive for *V. germanica* wasps when tested against a negative control (Lukas et al. 2020). However, both species showed the highest attraction when presented with a combination of the cues, which demonstrates that multi-modal stimuli are most important in finding flowers of *H. helix* for both *V. germanica* wasps and *C. hederae* bees.

Although bees and wasps differ in how they integrate floral signals in their foraging on *H. helix*, they respond to a similar set of physiologically active compounds in GC-EAD experiments, such as phenylacetaldehyde, acetophenone, 2-phenylethanol, (*E*)-*β*-ocimene, 4-oxoisophorone, and dihydrooxoisophorone (Lukas et al. 2020). However, in their antennal responses to the nitrogen-containing compounds, only the bees responded to 1-nitro-2-phenylethane and to 2-aminoacetophenone identified in *H. helix* (see Fig. 3 and Table 1), whereas only wasps responded to phenylacetonitrile (Lukas et al. 2020). Bee-specific responses were recorded also for the benzenoid 4-methylanisole. This compound attracts the oligolectic bee *Protodiscelis palpalis* (Colletidae), to their *Hydrocleys martii* (Alismataceae) host plants (Carvalho et al. 2014), as well as the fig wasps *Ceratosolen gravelyi* (Agaonidae) in the mutual interaction with *Ficus semicordata* (Moraceae; Chen et al. 2009). 4-Methylanisole might also be involved in host finding of *C. hederae*, but it was not included in the synthetic mixture tested in our study. The mixture was attractive when offered against a solvent control, but not equally attractive as the natural scent, which indicates that one or more behaviourally active compounds are possibly still missing in the synthetic mixture. Those could be compounds that were emitted in trace amounts and below the detection limit, such as was potentially the case for 4-methylanisole and 1-nitro-2-phenylethane.

Overall, most of the identified floral compounds of *H. helix* are widespread floral volatiles (Knudsen et al. 2006). The most abundant compounds 4-oxoisophorone, (*E*)-linalool-oxide furanoid, and acetophenone are also abundant in the floral scent bouquets of various other plants (Knudsen et al. 2006), where they are partly involved in signaling to other *Colletes* species. For example, (*E*)-linalool-oxide furanoid and linalool attracted males of *C. cunicularius* to *Daphne mezereum* (Thymelaeaceae) (Borg-Karlson et al. 1996). Linalool is also produced in the mandibular gland of seven *Colletes* species (Bergström and Tengö 1978) and functions as mate attractant pheromone in *C. cunicularius* (Borg-Karlson et al. 2003). Such common floral volatiles can attract the bees not only to *H. helix* but also to other flowers. However, linalool and its related metabolites occur in different enantiomers, whose specific ratios can mediate highly

specific interactions (Raguso 2016). The weak attractiveness of the synthetic mixture tested here on *C. hederae* might be due to naturally occurring enantiomers of *H. helix* not being the ones used in the behavioral experiments with synthetics.

Colletes hederae bees mainly restrict their visits to *H. helix* inflorescences (Westrich 2008) but given that they also visit a small set of other plant species, such as *Solidago canadensis*, it would be interesting to compare the scent bouquets of all of these species to determine whether they share attractive compounds. *Calluna vulgaris* (Ericaceae) and *Aster tripolium* (Asteraceae), the hosts of the closely related sister taxa *Colletes succinctus* and *Colletes halophilus*, are further good candidates for comparison, because individuals of these sister taxa switch sometimes to the preferred host plant of one of the other species (Kuhlmann et al. 2007; Müller and Kuhlmann 2008). The floral scent components of these plant species were already compared by Vanderplanck et al. (2017), but the identified compounds contained a high proportion of contaminants and differed greatly from the floral compounds identified by Lukas et al. (2020). Beside floral cues, Vanderplanck et al. (2017) also investigated whether these plant species have similar pollen characteristics that might explain the restricted pollen-collection behavior. They found that, while the pollen of *H. helix* and *C. vulgaris* contain large amounts of non-volatile β-sitosterol and δ5-avenasterol, *A. tripolium* contains other sterols. However, it is not known whether sterols play any role in the foraging behavior of bees, although these non-volatile compounds might act as gustatory signals after the bees have been attracted to the flowers by their visual and olfactory cues.

In conclusion, we showed that *C. hederae* bees respond physiologically and behaviorally to various floral scent volatiles emitted by *H. helix*, and that visual cues are equally attractive as olfactory cues. The integration of different floral-cue modalities in the bee's search image likely enables the bees to be highly effective in host finding. These findings are in congruence with previously studied *Vespula* wasps foraging on *H. helix* and various pollination systems that demonstrated that multi-modal stimuli are most important in finding host flowers. However, the relative importance of olfactory versus visual cues and specific antennal responses to floral volatiles can vary between pollinating species. Comparative studies about pollinators visiting different host plants or plant species that are visited by several pollinators can increase our knowledge about species-specific versus generally observed trends in plant–pollinator interactions.

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Declarations

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