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Attractant Activity of Host-Related Chemical Blends on the Poultry Red Mite at Different Spatial Scales

Thomas Auffray¹ · Alfonsina Arriaga-Jiménez^{1,2}  · Adrien Taudière¹  · Laurent J.-M. Roy³ · Benoît Lapeyre¹ · Lise Roy¹ 

Abstract

Many blood-feeding arthropods use volatile organic compounds (VOCs) to detect their vertebrate hosts. The role of chemical interactions in mediating the behavior of hematophagous insects and ticks has been investigated before but remains poorly understood in hematophagous mesostigmatic mites. The poultry red mite *Dermanyssus gallinae* is an obligatory blood-sucking mesostigmatic mite that feeds on birds and causes damage in poultry farms. We characterized the attractive response of *D. gallinae* to candidate VOCs previously reported from the odor emitted by living hens. We performed *in-vitro* choice-test bioassays as well as semi-field and field trials using baited and unbaited traps, in the presence and absence of hens. Among different tested combinations of VOCs, a blend of 5 VOCs (mix1.0) was significantly attractive to our reference population of *D. gallinae in vitro*, whereas the same individual compounds tested alone were not attractive. Ammonia was attractive on its own and increased the mix1.0 attractiveness. The attractiveness of mix1.0 was confirmed at ‘natural’ spatial scales in the absence of hens both at the lab and on the farm that provided the reference population. The presence of hens inhibited the mix1.0 attractiveness. The attractive power of mix1.0 was not found in other farms. This research is an important step to advance our understanding of host-parasite interactions in hematophagous mesostigmatic mites and paves the way for developing alternative control tools against *D. gallinae* by interfering with chemical interactions. Moreover, it underlines the importance of assessing kairomonal activity on different pest populations when developing attract-and-kill systems.

Keywords Kairomone · Volatile organic compounds · Ammonia · Hematophagous lifestyle · Host-parasite interactions · Field trials

Introduction

In blood-feeding arthropods that do not live on hosts, part of their life is devoted to finding a suitable vertebrate host for feeding and, thus, completing their life cycle (Balashov 1984). Vertebrates provide numerous sensory cues used by hematophagous arthropods to remotely identify, detect and locate suitable hosts (Takken and Knols 2010). Many blood-feeding arthropod species are attracted by odors emanating

from the body of hosts, including via the breath, and detect these semiochemical compounds through a sophisticated olfactory system (Logan and Birkett 2007; Takken and Knols 2010). These semiochemical compounds are part of kairomones, volatile or mobile substances released into the environment by an emitting species (here, the host), triggering a behavioral response in a receiving species (here, the hematophagous arthropod) and providing a benefit to the latter (here, resource acquisition) (Klowden 2013). In contrast, the interaction adversely affects the host (Logan and Birkett 2007). Detection by the hematophagous arthropod of kairomones emitted by the host generally results in a source-directed pathway, thus acting as a tactic attractant (Miller et al. 2009). However, in some cases, kairomones act as kinetic attractants, inducing activation of the arthropod without necessarily generating a source-directed movement (e.g., host odors on triatomine bugs at certain times of their life cycle, Guerenstein and Lazzari 2009). The chemical

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interactions between hematophagous arthropods and their vertebrate host are all the more complex as VOCs interact with each other and can change from attractive to repellent depending on their concentration. For instance, carboxylic acids, lactic acid, ammonia and carbon dioxide emitted by the sweat and skin of their human host play a kairomonal role in mosquitoes with synergistic and antagonistic interactions (Smallegange et al. 2005; van Loon et al. 2015). Inhibition of the attractiveness of the blend has been reported with specific concentrations of carboxylic acids, inhibition lifted by the addition of carbon dioxide (van Loon et al. 2015). In hematophagous triatomine bugs, ammonia has been shown to be attractive to individuals depending on physiological status, suggesting a parsimonious use of a single signal: ammonia acts as a kairomone for hungry bugs and as an indicator of refuge for satiated individuals (Taneja and Guerin 1997).

The physiological and behavioral basis of interspecific chemical interactions involving arthropods is relatively well known in insects, much less so in other arthropods, whose molecular chemosensory systems are known to be fundamentally different (Missbach et al. 2014), especially in arachnids (Vieira and Rozas 2011). Commonalities in the behavioral responses to odors between hematophagous insects and hematophagous Acari are expected, resulting from likely evolutionary convergence events under very particular similar pressures. About hematophagous arthropods, a large bulk of the research was published on odor information processing in insects (Diptera: mosquitoes, sandflies; Heteroptera: triatomine bugs, bed bugs; e.g. Taneja and Guerin 1997; Logan and Birkett 2007; Guerenstein and Lazzari 2009; Takken and Knols 2010). It is unclear to what extent the different stages of information processing differ and converge between hematophagous Insecta and Acari. Studies on chemical interactions in systems involving non-insect hematophagous arthropods have so far focused on ticks, while systems involving hematophagous mites remain relatively understudied (Sonenshine 2004; Carr and Roe 2016). Recently, we showed that the mite *Dermanyssus gallinae* (De Geer, 1778) (Acari: Mesostigmata) was indeed attracted to hen odor in small-scale behavioral measurement devices (El Adouzi et al. 2020).

In addition to the structural differences in molecular chemosensory systems between the hematophagous Insecta most studied so far (mosquitoes, triatomine bugs; all winged) and the hematophagous Acari (mites and ticks; all apterous), the winged or unwinged character is likely to substantially affect the chemosensory interactions *in natura*. Unlike flying arthropods, which are naturally able to direct their path directly toward a source of odor, crawling arthropods such as mites cannot directly follow an odor trail. They face difficulties related to the three-dimensional structure

of the substrates they walk on. For example, in a henhouse, mites must successively follow vertical and horizontal paths formed by the elements of perches, nesting boxes or cages,

to reach a host that emits a more or less radiant plume of odor. Therefore, to reach their host, the mites must move sometimes towards the gradient of odor, but also backwards or perpendicularly. This makes it unlikely that simply orientating attraction dominates in Acari. In ticks, the activating potential of CO₂ has been shown to outperform the attractive orientating potential in olfactometers and strong interactions were found between the negative geotropism of the larvae and the attractive or repellent behavioural response at small spatial scales (a few centimeters in olfactometers and other lab systems, Zeringóta et al. 2013, 2020). Larger-scale field trials have allowed for refining the understanding of ticks' chemical interactions (Carnohan et al. 2017). To our knowledge, the studies that have focused on chemical interactions involving kairomones in mites have all been focused on the evaluation of mite orientation in small-scale behavioural tests (a few centimeters in olfactometers, Petri dishes, or specifically designed mazes; Jalil and Rodriguez 1970a, b; Pernal et al. 2005; van Wijk et al. 2011; Eliash et al. 2014; El Adouzi et al. 2020). Therefore, the topological constraints associated with the crawling character of these small animals at larger scales were probably ignored.

The Poultry Red Mite (PRM) *D. gallinae* (Parasitiformes: Mesostigmata: Dermanyssoidea) is a hematophagous mite species that feeds on birds (Wood 1917) and is a serious pest for the egg industry worldwide (Valiente Moro et al. 2009; Sparagano et al. 2014; George et al. 2015). The small number of effective acaricide products and the growing consumer interest in food produced with minimal pesticide inputs make the development of alternative means of control urgent, notably by interfering in the chemical interactions between the mite and its host as part of attract and kill or push-pull strategies (Decru et al. 2020). Its micropredator lifestyle implies that PRM must repetitively seek out a suitable host during its life cycle (Lafferty and Kuris 2002). PRM spends most of the time at a distance from the host, aggregated with conspecifics in shelters located in the vicinity of the host. The development of PRMs from egg to adult includes one larval stage followed by two nymphal stages (Tucci et al. 2008). While the larva does not feed, the two nymphal stages require a blood meal to molt and reach the next stage, and female adults can feed up to 8 times for egg maturation (Wood 1917). Nymphs and adults contact the host only for rapid blood meals before returning to hiding places. To gain insight into how PRM interacts with its host, we need to investigate the determinants involved in host-seeking behavior and the underlying physiological processes.

Some studies have focused on the behavioral response of PRM to remote generalist sensory cues released by their

avian hosts, such as heat, vibration, moisture or CO₂ at small spatial scales. Minute fluctuations in temperature as low as 0.005 °C/s elicited activation in unfed PRMs in small arenas, which was interpreted as the recognition of a potential host passing nearby (Kilpinen 2001; Kilpinen and Mullens 2004). Vibrations are also considered an important activating host cue for PRM and a close relative (Kilpinen 2005; Owen and Mullens 2004). The response of PRM to CO₂ varies strongly with light, physiological status (freshly fed / fasting) and exposure dynamics (puff vs. continuous flow) (Kilpinen 2005; Koenraadt and Dicke 2010). The effect of exposure dynamics on the mite response to CO₂ suggests an ability of the mite to distinguish two different emission sources (host breath vs. conspecifics). In addition, Koenraadt and Dicke (2010) demonstrated the attraction of unfed PRMs to aged hen-feathers (sampled from litter) but not to fresh feathers (directly picked from the hen) in Y-olfactometers. These contrasting responses to odors emitted by hen feathers relied more likely on non-host cues possibly due to bacterial activity and the associated production of ammonia (NH₃) from the decomposition in the litter of uric acid excreted by birds (Schefferle 1965; Gałęcki et al. 2019). In a recent study, we showed that modifying the hen-host odor composition after ingestion of an additive feed produced inhibition of its attractiveness towards PRM (even a repulsive effect; El Adouzi et al. 2020). Lastly, we have patented an attractant blend (Roy et al. 2018) composed of fatty acids derivatives (aldehydes and carboxylic acids) recorded amongst the VOCs emitted by the whole body of living hens (El Adouzi et al. 2020). As fatty acids are known to be an attractant to hematophagous arthropods (Logan and Birkett 2007), including Parasitiformes (Osterkamp et al. 1999; Carr and Roe 2016), the compounds identified from hen odors in these studies may encompass kairomonal compounds used by PRM for host detection and location. Guerenstein and Lazzari (2009) highlighted that complex effects were mediated by general and specific host cues in a better-studied insect micropredator-host system (hematophagous bugs; Heteroptera: Triatominae). As the life traits of hematophagous bugs and PRM having particularly marked similarities (life at a distance from their host, strictly hematophagous feeding at all stages), such knowledge can be particularly valuable to support the study of red mites.

Unlike ticks, the olfactory orientation behavior of PRM is measurable in olfactometers and other small-scale systems (El Adouzi et al. 2020; Soulié et al. 2021). Nevertheless, it is not excluded that some attractive compounds have kinetic and non-tactic activity (*sensu* Miller et al. 2009) on a larger scale given the mite's mode of movement, strictly based on walking, with strong physical constraints related to its small size. In addition, the odorant landscape of poultry farms is likely to affect chemical interactions between PRM and its host. As a result of the decomposition by cohorts

of microorganisms of uric acid excreted by birds (Schefferle 1965), ammonia (NH₃) constitutes an important part of volatile compounds in poultry farms (Moore et al. 2011), with atmospheric concentrations varying between and within farms depending on different factors, including litter quality and bacterial communities (Gałęcki et al. 2019). Some ammonia concentrations were shown to be attractant to PRM in 4-cm arenas (Masier et al. 2022). Since ammonia is an integral and ubiquitous part of the odorant landscape of poultry farms, this compound can be expected to have either a neutral effect or an activating activity (i.e., act as a kinetic attractant) rather than a tactic attractant effect on this pest species. However, if fine local variations allow the mite to adjust its path, ammonia could also provide a trail to a shelter (aggregates form under dry hen droppings) or even be part of kairomones, since previous studies on hen odors did not explore such kind of chemical compound. A better understanding of the role of VOCs emitted by the host, in the search for blood meals in the complex odorant landscapes—including ammonia—of the farms would allow major progress in our understanding of the host-micropredator interactions. Complete hen odors, as collected from the bird in headspaces, have been shown to be attractive to *D. gallinae* (El Adouzi et al. 2020), as well as some of their VOCs, in the form of a blend of synthetic compounds (Roy et al. 2018). Understanding how this blend of avian origin acts on the behavior of the mite at different spatial scales and under farming conditions would represent a valuable addition to the knowledge on remote host sensing in mesostigmatic hematophagous mites. Furthermore, such information may help interfere with host-pest chemical interactions, representing a promising and environmentally friendly method to control mesostigmatic pests.

In this work, we analyzed the role of various host-emitted VOCs in the attractive response of PRM, both in laboratory and field conditions with a focus on one specific population infesting a laying hen farm in France. We investigated the attractant activity of a few aldehydes, carboxylic acids and one alcohol (all known to be emitted by the hens, Roy et al. 2018) as well as ammonia (NH₃) on the PRM behavior by measuring the attractive response of the mites collected in this focus farm using a choice-test bioassays. Knowing that several of the VOCs that make up hen odor and ammonia have been shown to be attractive to hematophagous insects, we assume that the former can play a classic kairomonal role and that the latter, ubiquitous, can interfere in the chemical interactions between the mite and its host. In order to best identify the attractant activity of the VOCs emitted by the host, we tested the compounds alone or combined in blends varying in proportions and/or stereoisomers in the presence or not of ammonia *in vitro* at small spatial scales. In order to evaluate the kairomonal role at 'natural' spatial scale, we tested the attractiveness of the blend identified as the most

attractive *in vitro* by assessing the abundance of mites caught on specially designed baited traps. The effect of distance, scent bait and their interactions were studied in the laboratory at a medium spatial scale (semi-field experiments). The ability of odorant blends to attract mites under farming conditions was evaluated using baited and unbaited traps in the field in different layer farms, including the focus farm from which the tested mites were collected, in the absence or presence of hens.

Materials and Methods

Mites for Lab Experiments

Poultry red mites were collected from an infested laying hen facility (focus farm, coded as DIN) in Pierrelatte (Drôme, France) and kept in Ziploc bags stored at 17 °C in a climatic chamber. Only adult females starved for 5 to 25 days were used for choice tests and semi-field experiments.

Choice Tests in Y-tube Olfactometers

Experimental Device

We used the same olfactometric test system with glass Y-tube olfactometers (central arm 150 mm long; lateral arms 200 mm, angle between arms 45°, internal diameter 40 mm) as in El Adouzi et al. (2020) to test the olfactory response of PRM. Air pushed by a vacuum pump (Laboport N86 KN.18, KNF Neuberger GmbH, Freiburg, Germany) was passed through activated charcoal and distilled water and then split into two equal air streams adjusted at the rate of 120 mL/min with rotameters (Swagelok, model VAF-G2-01 M-1-0, USA). Each airstream was then passed through a glass jar (2.5 L), where it was either loaded with an odorant stimulus (“test” airstream) or left as pure air (“control” airstream), before being introduced into the lateral arm of the olfactometer. The airflow rate was controlled every five bioassays using a Restek ProFLOW 6000 electronic flowmeter. A single adult female mite was inserted into the central arm using a fine brush. The female could crawl freely and choose either airstream for a duration of observation not exceeding 10 min. A choice was recorded when the female crossed a decision line in either lateral arm 150 mm from the Y junction and stayed there for at least 1 min. Females that remained motionless from the start of the test, did not reach the decision line, or came out of the olfactometer within the time delay were recorded as having made “no choice”. A clean olfactometer was used for each new mite tested and was then rinsed with 96% ethanol and air-dried before being reused. Test and control airstreams were switched between the two lateral arms of the olfactometer at each assay. Blanks

(two “control” airstreams) were alternated with tests to confirm the absence of any side bias in the setup. A minimum of 30 females were tested for each comparison.

To load an airstream with VOCs under test, twenty microliters of either single or blended VOCs were applied in a diffuser made of 200- μ L glass tubing containing the VOC or the blend that was inserted in a 1.5-mL glass vial sealed with a silicon/PTFE cap and pierced with a 4-cm long capillary tube made of fused silica (deactivated GC column, internal diameter 0.53 mm). A similar process was used to load an airstream with NH₃ except that a glass capillary tube was used instead of a deactivated GC column piece and the blends containing this compound were never made directly in their liquid form. Indeed, in order to avoid deleterious interactions in the liquid forms of the VOCs and the NH₃ solution, the mix9 (see below) was only made in air, by placing side by side two diffusers, one containing 20 μ L of mix1.0, the other 20 μ L of the NH₃ solution. An empty diffuser was used in the controls.

VOCs Tested Alone or Combined in Blends

To test the attractive response of PRM to hen-emitted or environmental single compounds and their blends, we chose nine VOCs among those typically emitted by the hens according to the results supporting the patent ID WO2018109417A1 (Roy et al. 2018), and one hydrogen nitride (NH₃) that is an integral part of the odorant landscape of poultry farms (see the detailed listing in Table 1; Roy et al. 2018; El Adouzi et al. 2020; Moore et al. 2011; Gałęcki et al. 2019). The six following VOCs and NH₃ were tested alone in choice tests: R-1-octen-3-ol, octanal, nonanal, (*E*)-2-nonenal, octanoic acid, nonanoic acid. The same compounds were assessed in combination in seven different equivolumic blends, prepared by mixing 5 or 6 VOCs in equal volume (mix1.0 to mix7; see Table 1). For example, to prepare 500 μ L of a 6-compound blend, we took 100 μ L of each compound, poured them into a vial, and mixed the solution carefully before taking the 20 μ L for each diffuser. Three other VOCs (1-octenol, 1-nonanol and 2-octanone) were only tested together in a single equivolumic blend also containing 5 of the 6 previously cited VOCs (mix8; Table 1). Because the olfactory systems of some arthropods can discriminate between stereoisomers (Leal 2004), i.e., different spatial configurations of the same chemical compound, we added the S-1-octen-3-ol in the list of VOCs tested in two blends (one equivolumic blend and one ratio-specific blend, see below). The respective role of the different VOCs in blends that exhibited attractiveness to PRM was established by comparing PRM response to the different blends and individual VOCs. The attractive blend with the smallest number of constituents was used to test both the effect of changing the ratio of VOCs (mix1.1 to mix1.6; Table 1) and the effect

Table 1 Composition of blends tested in choice-test bioassays. Numbers indicate how many measures (volume) of each volatile compound must be mixed to reach the proportion of each tested blend (one blend per line). For example, mix1.0 receives 5 ones because it is a blend of 5 compounds in equal parts. Given the very different nature of ammonia, we have chosen to avoid mixing it with other compounds in liquid form. The blend containing ammonia (mix1.0 +NH₃) was therefore made in air, by placing two diffusers side by side, one containing the mix1.0 and the other the ammonia solution. Blends with different ratio of VOCs and blends with stereoisomer variants were all derived from the mix 1.0 composition. In the blend mix1.6, the VOC ratio corresponds to the relative abundance observed in the hen odor (cf. patent ID #FR3060258A1). Concerning 1-octen-3-ol, a compound that has two spatial configurations, R-1-octen-3-ol was tested in all blends, and the S-1-octen-3-ol form was tested by using the equivolumic mix1.0 iso blend and the mix1.2 iso blend. ^a denote compounds tested alone in choice-test bioassay

Tested VOCs	Blend ID	R-1-octen-3-ol ^a	S-1-octen-3-ol	Octanal ^a	Nonanal ^a	(E)-2-nonenal ^a	octanoic acid ^a	nonanoic acid ^a	1-octenol	1-nonanol	2-octanone	Ammonia (NH ₃) ^a
Blends with equivolumic ratio of VOCs	mix1.0	1		1	1	1						
	mix2			1	1	1	1	1				
	mix3	1		1		1	1	1				
	mix4	1			1	1	1	1				
	mix5	1		1	1	1	1	1				
	mix6	1		1	1	1	1					
	mix7	1		1	1		1	1				
	mix8	1		1	1	1		1	1	1	1	
Blends with different ratios of VOCs	mix1.1	0.1		1	1	1		1				
	mix1.2	1		1	1	10		1				
	mix1.3	1		1	1	1		10				
	mix1.4	1		10	1	1		1				
	mix1.5	1		1	10	1		1				
	mix1.6	1.1		2	88.1	5.4		3.4				
Blends with other enantiomers	mix1.0 iso		1	1	1	1		1				
	mix1.2 iso		1	1	1	10		1				
Blend VOC + NH ₃	mix1.0 + NH ₃	1		1	1	1		1				5

of substituting the enantiomeric form (R to S) of the 1-octen-3-ol (mix1.0 iso and mix1.2 iso; Table 1). A seventeenth blend consisted of the most attractive VOC blend plus NH₃. The synthetic VOCs (> 95% purity) as well as ammonia hydroxide aqueous solution (28.0–30.0%) were all obtained from Sigma-Aldrich Co. Ltd. (St. Louis, MO, USA).

In order to estimate the evaporation loss rate in our diffusers, we weighed the contents of 5 diffusers stored at room temperature and containing mix1.0 using a precision scale (Precisa, LS-120 A SCS), at two different times for each: just after the introduction of liquid into the diffuser (T0) and 41 or 43 days (T + 41 or T + 43) later for four diffusers, at T0 and T + 101 days for the fifth diffuser. This was done at different periods of the year.

Semi-Field and Field Trials

Design of Traps

Preliminary tests have shown that it is almost impossible to trap PRM simply using substances (glue, grease) because of its close and permanent relationship to the substrate (the mite moves by walking, constantly probes the substrate and stops its path abruptly when it comes into contact with the tested substances). We have therefore designed and developed electric traps equipped with headspace to concentrate the VOC emission (attractive function) close to a double track of electrified metal allowing to electrify the mites passing over it (injuring function) and to project it on a glued plate (collecting function) (Fig. 1). The headspace was formed by a disposable plastic cup placed upside down on a perforated Bakelite (resin) plate to let the odor pass through the junction between the two. An odor diffuser of the same type as the one used in olfactometric tests was installed under the cup. The circuit board to electrify the mite was engraved under the Bakelite plate and was composed of two concentric circular tracks (see marks 10 and 11 in the Fig. 1) spaced 1 mm apart (mark 12). Each of the tracks was connected to a high voltage supply's positive or negative pole. The high voltage supply was a cheap electronic assembly made from the plans of an electric cattle fence. As with any commercially available fence energizer, the voltage delivered was greater than 20 kV, but was safe for the handlers as the energy involved was low. We used a single power supply in barn farms and the internal power grid in cage farms to supply all the traps (10 to 52) involved in each experiment. The sticky plate was fixed underneath the Bakelite circuit board prior to the start of the experiment and let in place for the duration of the experiment. The glued plate was removed at the end of the experiment and covered with a transparent food film in order to fix the mites. The plates from each trap thus treated were then transported to the laboratory for identification and counting of all trapped

mites using a stereomicroscope. In order to evaluate the effect of kairomonal blends, 60 identical traps were built by C. Belmont (Saint-Genis-Laval, France).

Evaluation of Trap Efficiency in Controlled Conditions (Lab 6-trap Experiments, Medium Scale)

In order to evaluate the effectiveness of the traps and to assess the spatial scale of mite—VOC interactions, we performed experiments at lab in an isolated room without any light source during the experiment according to the following 6-trap design (Fig. 2A), replicated several dozen times:

- On a PTFE (Teflon) plate of 1 × 1 m (1 m²), 6 traps including 3 baited and 3 control traps were arranged according to a random spatial arrangement: trap position and between-trap distance were randomly assigned at each round
- 200–300 PRM (all mobile stages mixed: protonymphs, deutonymphs and adults) collected from the DIN reference population (fasting for at least 4 days) were deposited in the center of the plate at the beginning of the experiment (T0)
- at T + 1 h (one hour after T0), the sticky plates were removed and the captured mites were counted.

To refine our understanding of hen-emitted kairomones and their interactions with NH₃, we tested the most attractive VOC blend (mix1.0), NH₃ alone and a mix of both (mix1.0 + NH₃). The experiments with 6 traps were repeated > 20 times per modality, with different spatial arrangements and at different times of the day (between 8:00 am and 6:30 pm). Knowing that PRM has nocturnal habits, we also completed the tests with series conducted overnights (but allowing fewer repetitions).

We also observed in preliminary tests that the mites reacted little when the ambient temperature was below 15 °C (most mites remained immobile at the release point).

Field Trials (Trap-based Experiments, Farm Scale)

In order to characterize the kairomonal activity of the blends identified as attractive to the PRM *in vitro*, we have conducted field experiments using our electric traps with a focus on sanitary empty periods in order to minimize competition with hen odors. The typical sequence of operation of a commercial egg-laying henhouse is as follows: entry of a flock of young laying hens (laying age), egg production for one year (flock period), removal of the spent hens, washing of the henhouse, and then a period of sanitary emptying (empty period) before entry of the next flock. Experiments were carried out in four commercial layer henhouses (including the focus farm DIN, the source of the DIN reference population)

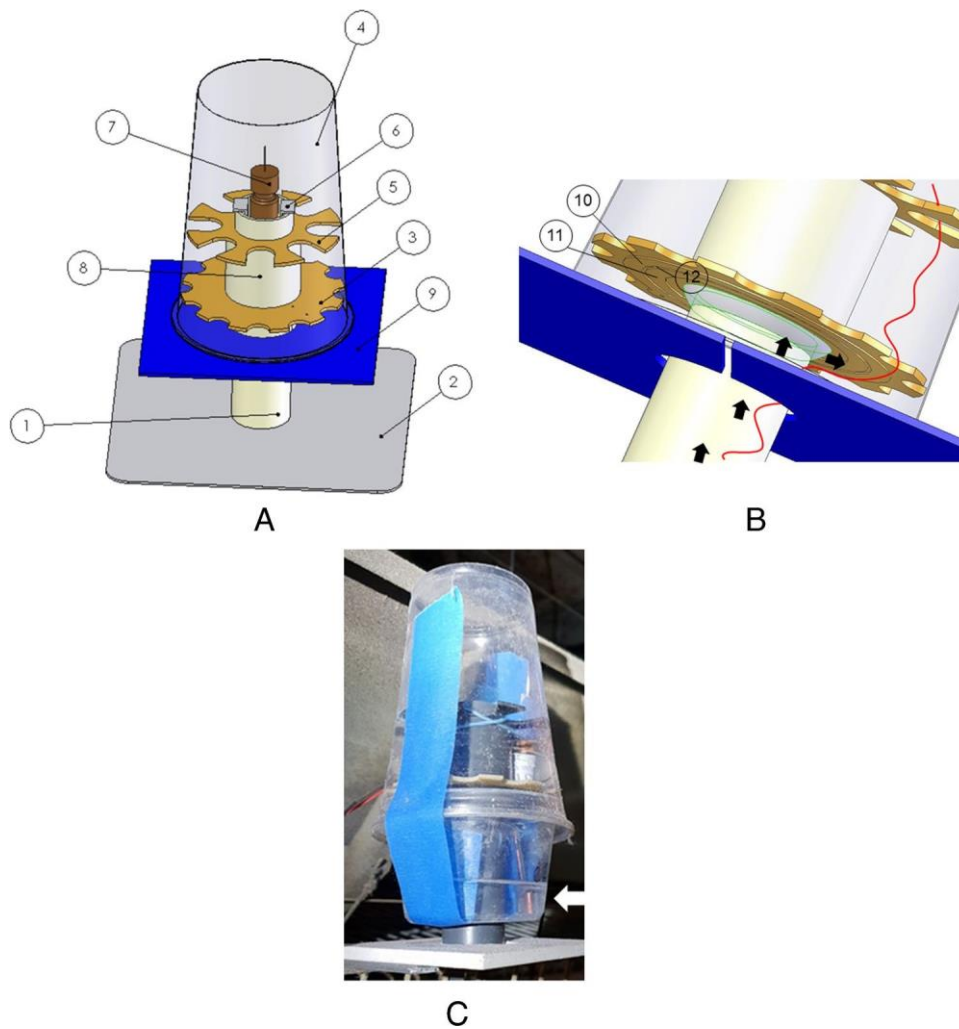


Fig. 1 Trapping system. **A.** Design of standard electric trap. 1, IRO25 PVC tube; 2, smooth plastic plate; 3, Bakelite printed circuit board (circular tracks underneath; injuring function); 4, transparent plastic cup (to form a headspace where VOCs accumulate; attractive function); 5, upper disc to stabilize the cup; 6, U-shaped support for diffuser; 7, diffuser containing the attractant blend (glass bottle closed by a cap with a silica capillary through which the VOCs escape; 8, spacer separating the discs (IRO32); 9, adhesive plate (collecting function). This part also contributes to the formation of the headspace, in combination with the cup. It was replaced by a circular plastic moat filled with silicone oil in a small number of trials to check whether mite-emitted pheromone could alter response to the baited traps (see C). **B.** Overview of the operation of electrocutting zone of the trap (injuring function for the mite, inaccessible to the operator

and the hen). The mite (black arrows) attracted by the VOCs (plume figured by a red line) that are emanating from the diffuser (located at the top) reaches two concentric copper circles by climbing along the cylindrical pillar. The circles were made of two copper tracks (10, external circle, 11, internal circle) spaced 1 mm apart (12, gap) and constituting part of an open printed circuit powered by a battery. The mite closes the circuit as it passes over the two tracks. This produces its immediate electrification and its projection on the sticky surface of the blue plate. The plate was collected at the end of the experiment and all the mites trapped were counted. **C.** Photograph of a trap adapted to avoid the possible effect of pheromones emitted by the trapped individuals by replacing the blue plate by a circular plastic moat filled with silicone oil (white arrow) maintained by tape

in the absence of hens, just after the removal of the spent hens and before cleaning and disinfection of the henhouse, at the beginning of the sanitary empty period between two flocks. In addition, we also conducted a few experiments in the presence of hens, including the focus farm DIN (source of the DIN reference population) and another one in order to determine to what extent competition with odors and other stimuli emitted by the hens (heat, CO₂) affects the activity

of the blend (Table 2). The level of PRM infestation in each henhouse under test was consistently verified beforehand by using water trapping (see Mul et al. 2017).

Consistent with our lab medium-scale preliminary experiments, mites in our field experiments were active and easily trapped when temperatures recorded in the henhouse were between 15 °C and 25 °C. Two experiments failed to trap mites during sanitary empty periods in winter (between 5

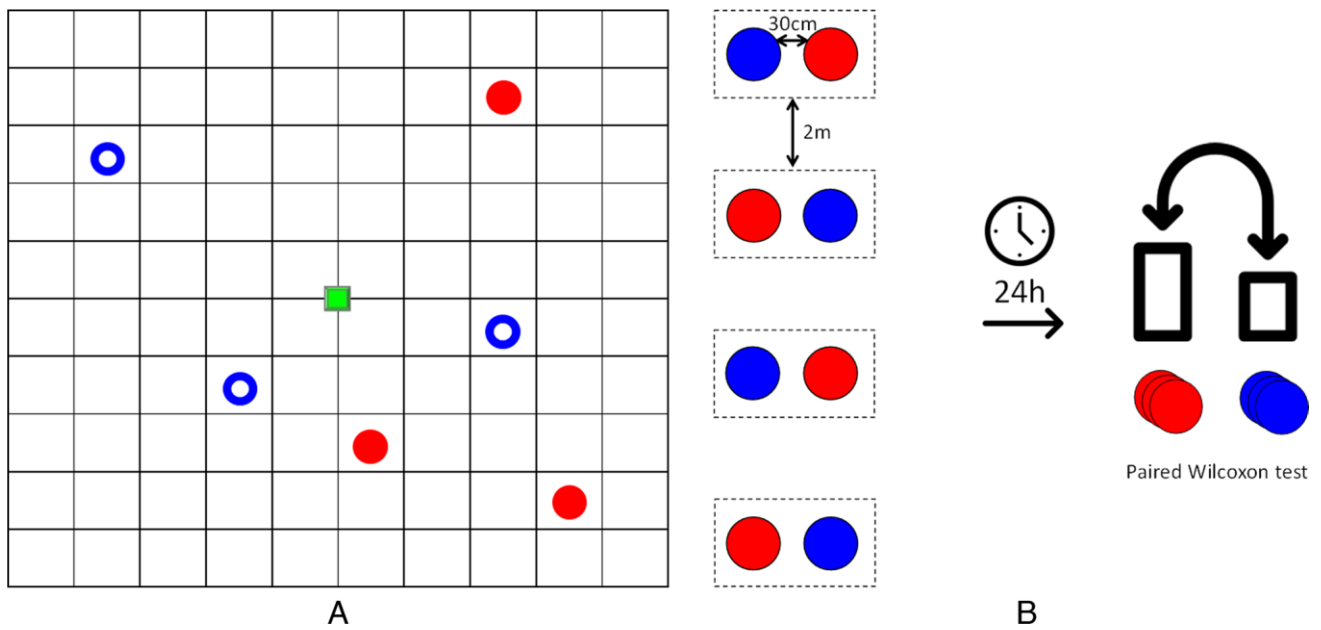


Fig. 2 Illustration of the trapping designs. **A.** Lab 6-trap design. Six traps were positioned randomly on a 1×1 m Teflon plate. Green square, mite release point (central); open blue circles and solid red circles, position of control and test traps (resp.) assigned by drawing lots out of 100 possible positions (open squares). **B.** Field paired traps design. *Left*, relative positioning of the traps. The position of control

and baited traps was alternated between two successive pairs (control on the left and baited on the right, then reverse) along the henhouse. *Right*, mite count values on the plates were analyzed by pair. The distance between traps of the same pair was 30 cm, distance between two pairs, 2 m

Table 2 Information on commercial henhouses where experiments with pairs of baited test traps and unbaited control traps have been conducted. Test traps were baited with mix1.0 in all cases except for the series in italics (preceded by a+), where test traps have been

baited with mix9 (mix1.0 +NH₃). *N pairs empty*, number of trap pairs during the sanitary empty period, *N pairs flock*, number of trap pairs during the flock, (*in brackets*), number of traps usable at the end of the experiment if any have been damaged.

	City (county code)	Farm type	N pairs empty	N pairs flock
SPT	Saint-Pons-de-Thomièr (34)	cage	10	+26
FOU	Grenade (31)	cage	10	0
DIN	Pierrelatte (26)	barn	10(8) + 13	20
TES	Portes-lès-Valence (26)	barn	10	10

and 10 °C in the focused henhouse in January 2018; data not shown). In these experiments, the temperature of the henhouse dropped very abruptly after the hens were removed.

A minimum of 10 paired electric traps (one control and one baited) were installed in each barn henhouse as shown in Fig. 2B. In the barn henhouses, the traps were installed on the (slatted) floor, on either side of the perches. Traps and power supply cables were protected against the hens with cages and plastic sheaths when experiments were carried out during the flock in barn farms. In the caged hen buildings, the traps were installed along the egg belt, without any specific protection even during the flock since the trap positions were out of reach of the hens. The experiments were conducted at different times of the year, from winter to summer. The traps were set during the day in the afternoon to start the experiment (T0) and the plates were recovered at T + 18 h, encompassing one full night and a piece of daytime. Test traps were baited with the most attractive VOC blend (mix1.0) in all farms in the presence or absence of hens and with the same blend added with NH₃ in two of these farms (Table 2).

Entrekin and Oliver (1982) showed that crushed mites contained a pheromone that was very attractive to freshly fed individuals seeking shelter for post-prandial rest. We suspected that the message sent by this pheromone could blur the message sent by the kairomone and reduce the effectiveness of the baited trap past a threshold of trapped mites. In order to be free from potentially confounding effects of the pheromones emitted by the mite corpses fixed on the glued plate and maximizing the kairomonal attraction, the glued plate of the traps in two experiments was replaced by a silicone oil moat (VISCOSITY 100 CST, Sigma). A circular trough was formed by inserting a plastic smartcup (Smart Espresso, JMG, Basiglio, Italy) upside down around the trap pillar and we poured the silicone oil into the circular trough once the trap was installed in the henhouse (Fig. 1C). Consequently, electrified mites were immersed in silicone oil (previously tested neither attractive nor repellent) instead of just glued on a plate to prevent the diffusion of the pheromone into the air by the dead mites.

Statistical Analyses

All the analyses were performed by using R software (R Core Team, 2021; url: <http://cran.r-project.org/>). For olfactometric *in-vitro* bioassays, to determine whether the test airstream attracted mites, we conducted binomial tests based on the null hypothesis that the probability of scores for the test airstream or the control airstream is equal to 50%. “No choice” tests were excluded from the analyses.

The analysis of the scale of mite-VOC interactions and of the effectiveness of the traps was conducted as follows on results from our lab 6-trap experiments: Pairwise dis-

tances between traps in our dataset were calculated using the function *daisy* in the “cluster” package. We measured the effect of the proximity of a baited trap on the number of mites trapped in the control traps by constructing the following linear model: $nb_acari \sim dist_center + min_dist$, with *nb_acari* the number of acari (log10-transformed) in control traps, *dist_center* the distance of control trap to the center (mite release point) and *min_dist* the distance of control trap to the nearest baited trap. We measured the effect of modality (baited traps vs. unbaited traps as control), with 3 bait modalities (mix1.0, NH₃, mix1.0 + NH₃), and distance on the number of mites trapped using pairwise Wilcoxon tests (*pairwise.wilcox.test* function) with p-value correction by the Holm method. Each run tested one modality (e.g. mix1.0 + NH₃ as bait) and a given set of 6 distances. We conducted > 20 runs per modality.

To assess the effect of modality in the farm odorant landscape (on farms), the analysis of results from field experiments was carried out by paired samples Wilcoxon tests (*wilcox.test* function with *paired = TRUE*).

For all analyses, we considered as significant those tests for which $p < 0.05$.

Results

Attractant Activity of Host-emitted VOCs and Ammonia at Small Spatial Scales

In the *in-vitro* behavioral tests carried out with PRM collected from the DIN population, two blends out of the eight tested blends of VOCs mixed in equivolomic ratios (see Table 1) attracted significantly more PRMs than the control: the 8-VOC mix8 and the 5-VOC mix1.0 (which shares all VOCs with the former) (Fig. 3A). More PRMs chose the 6-VOC mix5 (which shares all VOCs with mix8) over the control, although the distribution was not significantly different from 50/50 ($p = 0.052$). When tested individually, the VOCs that compose the mix1.0 did not attract significantly more PRMs compared to control (no significant differences between responses to test or control arms of the olfactometer; Fig. 3B). Interestingly, the removal of any of the VOCs in mix1.0 (R-1-octen-3-ol, octanal, nonanal, (E)-2-nonenal and nonanoic acid) resulted in the loss of attractiveness since none of the other 5-VOC mixes (that share VOCs with mix5) were significantly attractant (Fig. 3A).

With VOC ratios that were not equivolomic, although more PRMs chose the test odor in all tests, only the blend with (E)-2-nonenal as a dominant constituent (mix1.2) was significantly attractant to PRMs (Fig. 3C). When the VOCs of mix1.0 were mixed in the proportions corresponding to that observed in chromatographic profiles of hen odors (mix1.6, Fig. 3C), PRMs did not choose the blend more than the control. Finally, the attraction of PRMs to blends mix1.0

and mix1.2 was lost when S-1-octen-3-ol replaced R-1-octen-3-ol (Fig. 3C).

In our tests to estimate the evaporation loss rate in our diffusers, the evaporation of mix1.0 generated, at room temperature, a loss of 0.029% (± 0.014) of the mass over periods of 41–43 days in 4 diffusers (replicates) and 0.560% over a period of 101 days in the fifth diffuser.

Effect of Kairomonal Blends and Distance on the Path of Mites at a Medium Spatial Scale

The 6-trap lab experiments conducted on our DIN reference population showed an interesting capacity of the traps to capture PRM (mean number of mites trapped within one hour during daytime in baited traps \pm sd = 20.1 ± 37.1 , in control traps = 7.4 ± 13.3 , out of 200–300 mites released). A significantly higher number of mites were trapped in traps baited with either of the three baits (mix1.0, NH₃, mix1.0 + NH₃) as compared to control traps (Figs. 4, 5). However mix1.0 alone affected the number of mites trapped in test traps less than other baits (62.5% of tests ($n = 24$) with more PRM in mix1.0-baited traps than in control traps vs 96.9% with NH₃ and 88.6% with Mix1.0 +NH₃).

We observed a strong tendency of PRM to move erratically in a space of a few tens of square meters, with many mites trapped in control traps (Fig. 6). A negative covariation between the number of mites trapped and the distance was recorded in both control and baited traps and the relative distribution of numbers of mites trapped by distance class was essentially the same between controls and tests (Fig. 6). The difference between the two middle classes

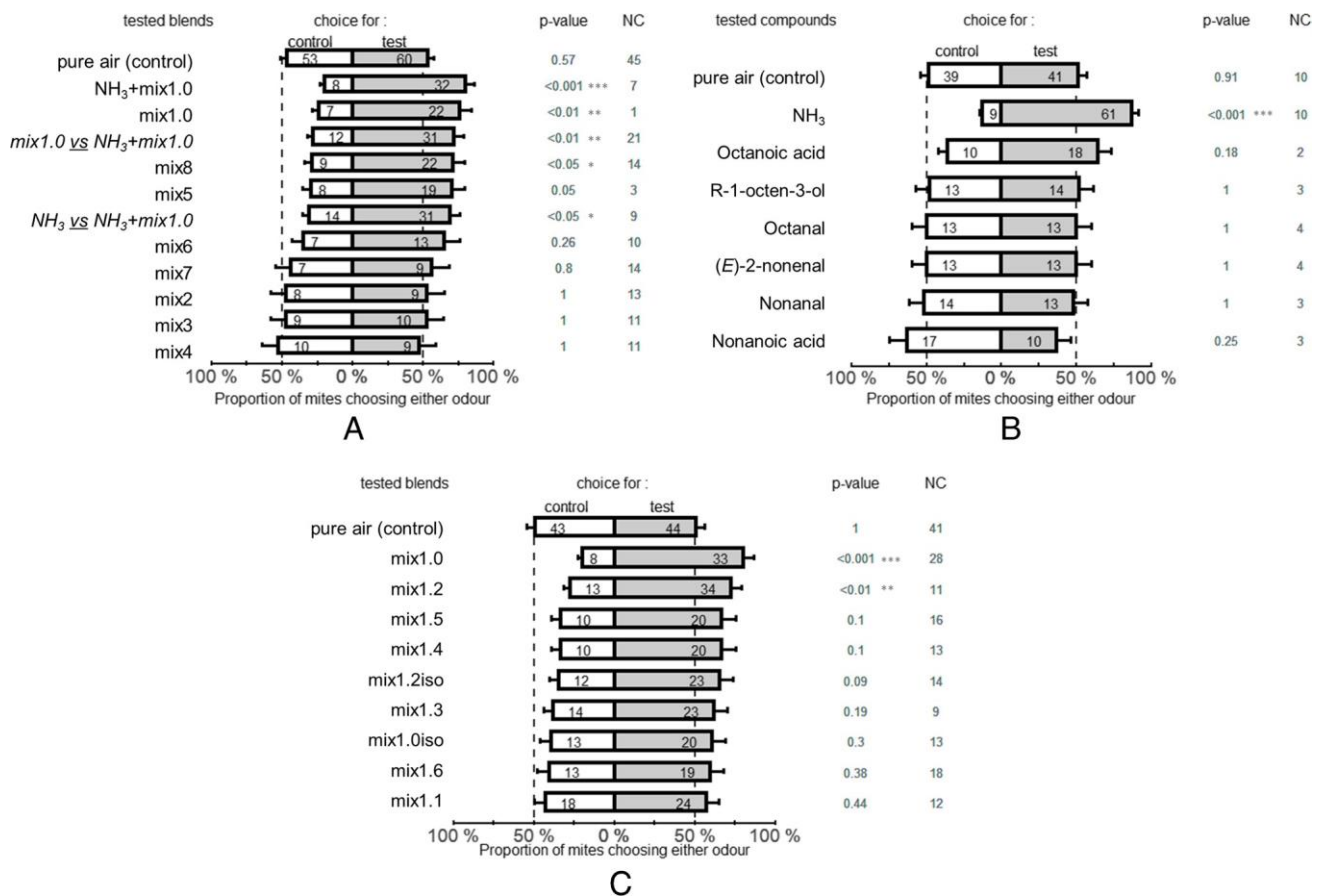


Fig. 3 Attractive responses of starved female PRMs choosing the test or the control in Y-tube olfactometers (DIN population). Tests involve (A) equivolomic blends of VOCs in the presence of NH₃ or not, (B) single compounds, (C) blends of VOCs with unequal ratios. The composition of each blend is indicated in Table 1. The white bars on the left represent the percentage of mites that chose the control branch (pure air) and the grey bars on the right the percentage of mites that chose the branch with the tested VOC(s). The indications on the right (tested blends or compounds) indicate the VOC(s) tested. In two cases in Fig. 3A, the test did not oppose a test branch to a control branch, but two test branches against each other, each traversed by air loaded with different groups of volatile compounds

(tested blends in italics). In both cases, the white bar on the left represents the percentage of mites that chose the compound indicated to the left of the term "vs." and the grey bar the compounds indicated to the right. The control modality in each figure indicates the choices made by the mites confronted with two branches with pure air. Numbers in the bars indicate the number of mites that chose either arm. NC denotes mites that did not choose either arm ('No Choice') and were excluded from statistical analyses. Asterisks indicate significant difference between test and control (or between the two tests in the two italic cases of Fig. 3A) using two-sided binomial test ($P=0.5$): *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ (only significant differences are marked)

(25–44 and 45–63 cm) was significant in the tests (significantly more mites trapped at 25–44 cm from release point than at 45–63 cm), not in the controls (similar number of mites trapped at 25–44 cm and at 45–63 cm from release point) (Fig. 6).

Similar results were observed in tests conducted at night in the dark, although with fewer replicates (data not shown). The above results therefore reflect the behaviour of this somewhat nocturnal mite, although the bioassays were conducted during the day.

Ability of Odorant Blends to Attract Mites in Farm Conditions

The traps generally trapped many mites on farms, at least when the ambient temperature was mild and when the infestation level was high (see below). The average number of mites trapped was highly variable between farms, but consistent with the level of infestation in the barn as estimated by careful visual inspection and water trap surveys. For example, in both DIN and TES barn henhouses, the

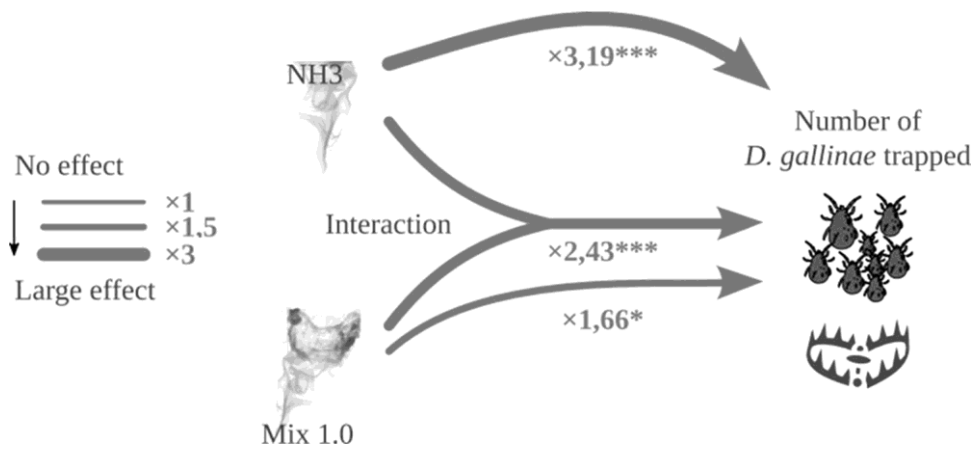


Fig. 4 Effect of the presence of mix1.0 and/or NH₃ on the number of mites trapped in the lab 6-trap experiments (DIN population). The picture summarizes the outputs from the following linear model: $nb_acari \sim dist_center + min_dist$, with nb_acari the number of acari (log10-transformed) in control traps, $dist_center$ the distance of control trap to the center (mite release point) and min_dist the distance of

control trap to the nearest baited trap. The thickness of the arrows is proportional to the effect. Pairwise Wilcoxon tests were conducted to assess the effect of modality (baited vs. control, with 3 bait modalities: mix1.0, NH₃, mix1.0 + NH₃) on the quantity of mites trapped. Asterisks indicate significant difference between modalities from these tests: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

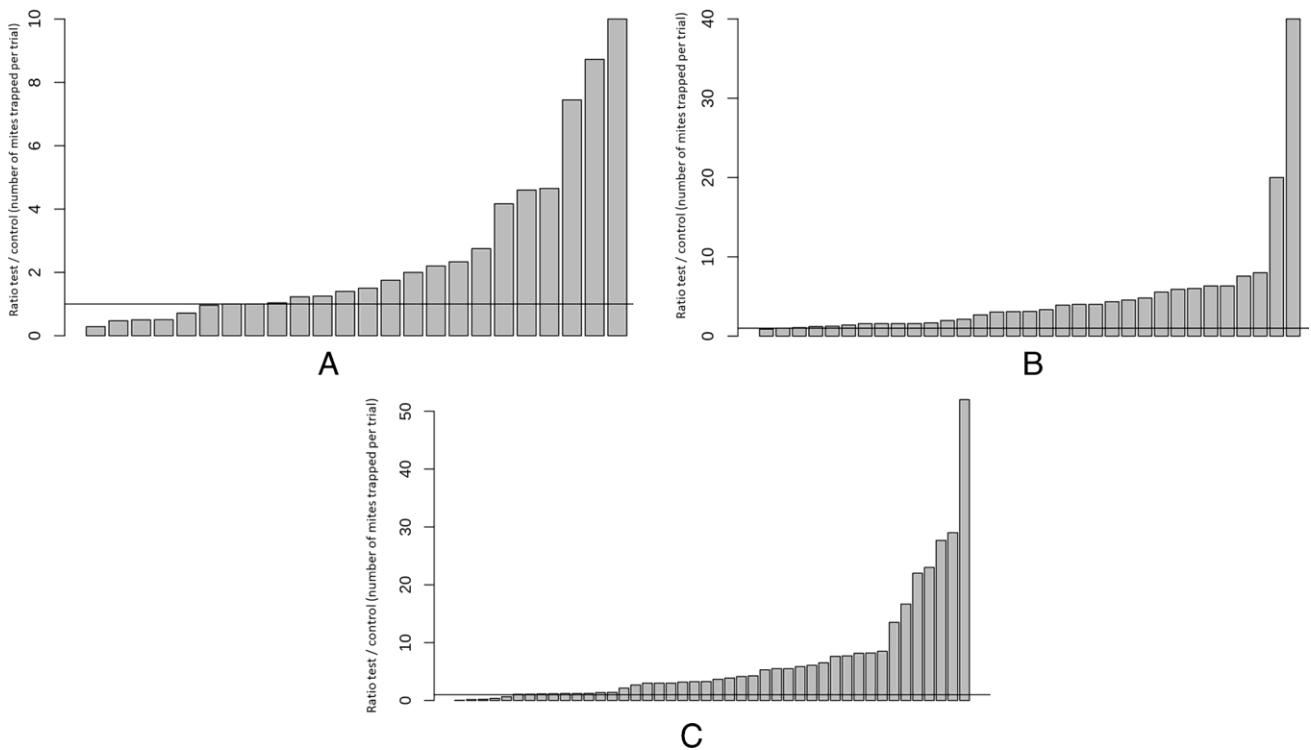


Fig. 5 Ratio of the number of mites trapped in test traps to the number of mites trapped in control traps (=sum of the 3 test traps or the 3 control traps) in our lab 6-trap experiments. One grey bar corresponds to one test (counts from the 6 traps used during a one-hour

period); a value greater than 1 (horizontal line) indicates more trapping by the test traps than by the control. **A.** mix1.0 ($n = 24$), **B.** NH₃ alone ($n = 33$). **C.** mix1.0 + NH₃ ($n = 44$)

infestation levels were very high and very low, respectively, in agreement with the recorded values (Fig. 7). The baited traps significantly trapped more mites than the unbaited

control traps in the absence of hens in the DIN farm during the sanitary empty periods (2 different experiments), i.e., the farm where we had sampled our reference population (Fig. 7). In all other farms as well as in the same farm during the flock, we did not get any significant difference between baited and control traps.

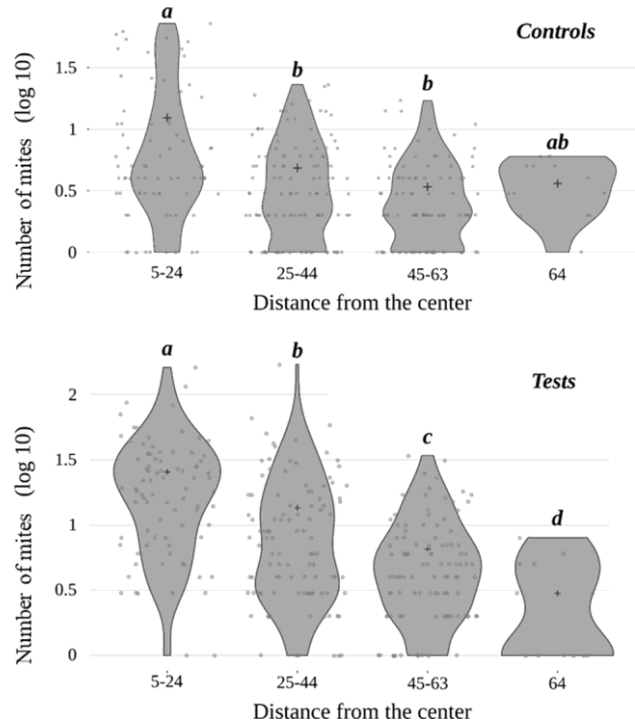


Fig. 6 Effect of distance in our lab 6-trap experiments (DIN population). The letters correspond to the results of the Wilcoxon pairwise test (threshold $p < 0.05$; Holm adjustment) and the black crosses indicate the average values. Tests include all baited traps (mix1.0 ($n = 72$), NH_3 ($n = 99$), mix1.0 + NH_3 ($n = 132$))

Discussion

Attractant Activity of Host-emitted VOCs at Small Spatial Scales

In this study, we characterized for the first time a candidate kairomone behaviorally active on PRM, the main pest in the egg-laying industry worldwide. Our results demonstrated that starved female PRMs sampled in one French farm were attracted by a complex blend of five synthetic VOCs, namely, R-1-octen-3-ol, octanal, nonanal, (*E*)-2-nonenal,

and nonanoic acid, combined either in equivolumic ratios or with (*E*)-2-nonenal as the major constituent. The addition of other compounds seems to not alter the kairomonal activity (1-octenol, 1-nonanol and 2-octanone, mix8) or slightly weaken it (octanoic acid, mix5).

The five compounds of the kairomonal blend seem to act synergistically as an attractant since the attractiveness of the equivolumic blend was lost as soon as one of them was removed and fully maintained or almost maintained when other hen-emitted VOCs were added (mix8, mix5). Our results are consistent with previous studies showing the ability of hematophagous micropredators to respond to a complex mixture of VOCs (Barrozo and Lazzari 2004a; Guerenstein and Lazzari 2009). Except nonspecific VOCs

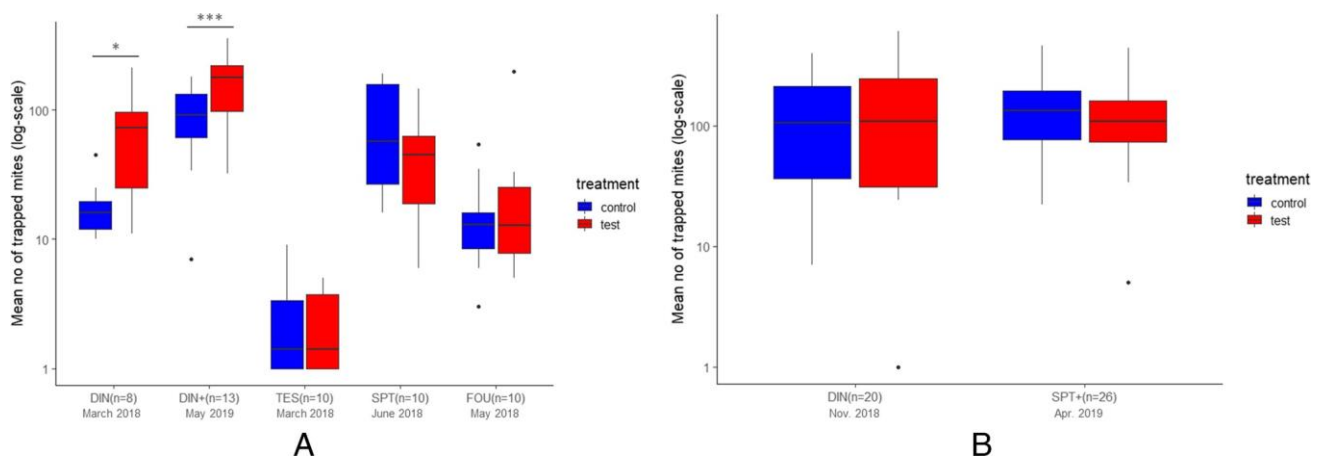


Fig. 7 Number of mites trapped during the field trial (trap-based farm-scale experiments). **A.** In the absence of hens (during the sanitary empty period). **B.** In the presence of hens (during the flock). The traps were arranged in test-control pairs as shown in Fig. 2B. The test traps were baited with mix1.0 alone (treatment = mix1.0) in all experiments except when a + follows the farm name: in this case, ammonia was added to the bait of the test traps (treatment = mix1.0+NH₃). n, number of trap pairs (one pair=one test trap+one control trap).

Asterisks above pairs of boxplots indicate significant difference between modalities using paired samples Wilcoxon tests: *** $p < 0.001$, ** $p < 0.01$ (only significant differences are marked). Note 1: The vertical axis is log scale in order to visualize results from hen-houses with contrasting levels of infestation (the level of infestation was high in all houses except TES). Note 2: The statistics are based on paired tests. This allows to measure differences that do not appear on boxplots

emitted by vertebrates, such as CO₂ or ammonia (NH₃), which elicit a response from virtually all hematophagous arthropods (Guerenstein and Lazzari 2009), the specific attractive response to a combination of host-emitted VOCs generally exceeds that observed with single VOCs alone. For example, blends of host-emitted VOCs have been shown to be attractive to the triatomine bugs *Triatoma infestans* and *Rhodnius prolixus*, the cimicid bug *Cimex lectularius* and the tick *Boophilus microplus* while their individual components did not evoke any attraction (Osterkamp et al. 1999; Barrozo and Lazzari 2004a, b; Singh et al. 2012; Guidobaldi and Guerenstein 2013). The search for a complex olfactory signature allows the micropredator to distinguish host cues within a complex chemical landscape, thus avoiding wasteful efforts directed towards a nonsuitable host (Lehane 2005; Guerenstein and Lazzari 2009). The recognition of a blend over individual VOCs is interpreted as the need for synchronous activation of suitable odorant receptors (termed as coincidence detection) followed by the integration of the produced signal in the neural network (Logan and Birkett 2007; Bruce and Pickett 2011).

The contrasting response to blends depending on stereoisomers of the same molecule suggests a high specificity of PRM odorant receptors to the kairomonal VOCs. The attractive response of PRM was lost when S-1-octen-3-ol was substituted for R-1-octen-3-ol, the predominant stereoisomer of 1-octen-3-ol in nature (Kline et al. 2007). A similar specificity to the isomer was previously shown in some mosquitoes with R-1-octen-3-ol vs R-1-octenyl-3-ol and the triatomine bug *Triatoma infestans* larvae with L-Lactic acid vs D-Lactic acid (Kline et al. 2007; Barrozo and Lazzari 2004b). Once more, our results are consistent with results on distantly related hematophagous insects and this finding highlights the crucial role of the kairomonal signature in the lifestyle of hematophagous micropredators.

The attraction-type behavioral response of PRM was mainly observed with equivolumic ratios of the different VOCs in the blend and was lost when we tried to mimic “natural” ratios. The relative proportion of VOCs in a blend was demonstrated to play a crucial role in host-odor recognition in some herbivorous insects (Bruce and Pickett 2011). We expected that applying the relative proportions of VOCs measured in hen odors (El Adouzi et al. 2020) to the kairomonal blend would lead to an enhancement—or at least the maintenance—of the attractive response. The failure of our rough hen-mimicking blend mix1.6 to attract mites may be due to poor quantitation data generated by the SMPE fibers (Solid phase microextraction; Romeo 2009) used to collect the odor. While it was supposed to mimic the natural proportions of VOCs in hen odor, it probably deviates too far from them.

Host-emitted VOCs at Higher Spatial Scales and Mite’s Odorant Landscape

At medium spatial scales (lab 6-trap experiments), the movement of hungry adult females appears to be primarily governed by chance with odorant blends intercepting mites in their path. Indeed, mites did not appear to be constrained by the distance between 25 and 63 cm based on the amounts of mites trapped in the control traps. This suggests that host-seeking mites are active in all directions and will climb up the trap’s pillar and then walk the electrical circuit of any trap, baited or not, if one is in their path. Moreover, the higher number of individuals trapped and the effect of distance between 25 and 63 cm in baited traps shows that the attractiveness of the blends affects their path very locally. The olfactory bait increases the probability that the mite will walk the electrical circuit, with the olfactory signal likely affecting their erratic path only on passes very close to the odor source.

Furthermore, we identified a synergistic effect of ammonia (NH₃) with our mix1.0, a blend made of fatty acid derivatives. In our medium spatial scale experiments, the attractive activity of our basic kairomonal blend mix1.0 was exceeded by ammonia alone, but their interaction had a significantly positive effect. Furthermore, in olfactometer tests, the mites showed a significant preference for the extended kairomonal blend (mix1.0+NH₃) over NH₃ alone when both options were offered simultaneously. This demonstrates that adding ammonia to our blend increases its attractive power.

Smallegange et al. (2005) established a similar pattern in the mosquito *Anopheles gambiae*: ammonia is attractive on its own, whereas lactic acid and carboxylic acids are not, but their blend shows synergistic activity and contributes strongly to the orientation of the mosquito towards its human host. Although the hen itself may emit ammonia, this compound is ubiquitous in the habitats frequented by PRM in poultry houses and is a priori fully part of the typical odorant landscape of a foraging nidicolous avian micropredator.

Overall, the volatile compounds used by PRM to forage in poultry houses are ubiquitous. The five VOCs (R-1-octen-3-ol, octanal, nonanal, (E)-2-nonenal and nonanoic acid) included in the mix1.0 blend are all fatty acids (short chain carboxylic acids, aldehydes or alcohols), a class of VOCs commonly found in odors emitted by vertebrates (Syed 2015) and ammonia is emitted in large quantities from poultry manure and litter (Moore et al. 2011). Host remote recognition by hematophagous arthropods often relies on olfactory signatures involving multiple ubiquitous VOCs common to various host species rather than unique species-specific VOCs, with the synchrony of compound perception determining arthropod decision making more than compound identity (Guerenstein and Lazzari 2009; Syed 2015; Majeed et al. 2016). The use of ubiquitous VOCs has

the evolutionary advantage of allowing flexibility by being able to detect changing signatures with the same peripheral olfactory receptors and plays an important role in the evolution of host specificity. Such a strategy is also associated with low host specificity in many instances, as in soft ticks (family Argasidae), whose lifestyle is quite similar to PRM. These ticks are more specialized in off-host habitat than in host, feeding on very distant taxa as long as they share resting and roosting sites (Klompen et al. 1996). The off-host habitat specialist trait may also apply to PRM, as it has been recorded in at least nine different orders of birds and is capable of feeding on mammals, while remaining highly dependent on environmental factors such as farms vs. wild bird nests (Roy et al. 2009; George et al. 2015; Roy and Buronfosse 2011). Interestingly, the synergistic activity of ammonia in PRM could be explained by a role as a host habitat marker, rather than as a kairomone emitted by the host as in mosquitoes (Smallegange et al. 2005): ammonia perceived at the same time as the fatty acids of the host signature would signal the presence of a bird in a nesting context. The bird in this context is a priori not in a passing position but in a resting position (perched or laying eggs), a more appropriate situation for the meal of PRM because it limits the risk of crushing. In order to further our understanding of host-PRM interactions and its level of specialization, it would be interesting to test whether our attractant blend is a hen-specific signature, a bird-specific signature that PRM can distinguish from non-host mammalian odors, or a vertebrate-specific signature.

Ability of Kairomonal Blends to Attract Mites at "Natural" Scales

The significant differences found in the trapping data in the DIN farm during sanitary empty periods with both the simple blend mix1.0 and the expanded blend mix1.0 +NH₃ show that the small- and medium-scale responses (all conducted with mites from this farm) are well expressed at the natural scale. This suggests that the identified blends, similar to the hen odor, do play a kairomonal role. However, the fact that only the population from our reference farm (DIN) responded to this blend in the field trials suggests that the sensitivity of the mites is heterogeneous. Indeed, with the DIN population, the attractive response to the mix1.0 was significant in olfactometer tests, i.e., at very small scales under conditions where odor diffusion was regular, in lab 6-trap experiments, i.e., at medium spatial scales with uncontrolled airflow, and in *in-situ* experiments during the sanitary empty period, i.e. at large scales under farm conditions. The attractive response was null in this DIN population only in the field trials when hens were present. This may be easily explained by competition with the volatile compounds emitted by the hens themselves: natural hen

odors can be all the more attractive as they are associated with the CO₂ they exhale. On the other farms, we did not observe a significant large-scale attractive response under farm conditions, in either the absence or presence of hens.

The tested farms were not all configured the same (2 barn and 2 cage farms), so the comparison should be handled cautiously. The air flows are probably structured differently between building types and the access paths to the host for the mite are certainly different. However the differences in building structure do not explain the failure to attract more mites in baited traps than in control traps in farms other than DIN since such a failure occurred in another barn farm quite similar to DIN (TES) (as well as in another farm not presented here, JOU). In addition, we did not find any trapping failure in the two cage farms (FOU and SPT) control traps, showing that mites did reach the traps in both building types.

In short, in our study, a single population (DIN) exhibited a consistent, attractive response at all scales in the absence of hens while we did not detect any significant response from other mite populations in the field. However, we do not know how these other populations respond at smaller and medium scales. The lack of a detected attractive response to our synthetic kairomone could result from either strictly behavioral factors (unresponsive mites able to detect the VOCs but not using them to optimize their host search), or variations in sensory receptors, as previously shown in *Drosophila melanogaster* with respect to benzaldehyde (Rollmann et al. 2010). In the latter case, the lack of response detected in the field could be explained either by decreased average sensitivity in non-DIN populations or even by increased average receptor sensitivity, with a lowered attractive-repellent transition threshold. Indeed, as with mosquito kairomones (Bhasin et al. 2000), some higher concentrations of mix1.0 have repellent effect in PRM, at least at small scale as shown by our team in recent experiments (Masier et al. 2022). The concentrations received by the mite were not measured in any of our systems (Y olfactometers, electronic olfactometers, electric traps) and we cannot compare them with each other due to the diversity of diffusion modes (VOC-laden airflow, impregnated filter paper tape in still air, diffusion from a headspace, respectively). Nevertheless, because the transition thresholds between attractive and repellent concentrations of mix1.0 vary among PRM populations including DIN (Masier et al. 2022), it is plausible that the single concentration used here may be attractive to some but not all populations tested in the field. In short, the results of this study show that it is difficult to attract various populations of PRM in henhouses using traps baited with a single amount of mix1.0.

Whether they result from variations in receptor sensitivity or from downstream processes, differences detected in the field between populations likely come from variable ratios of locally attracted, indifferent and repelled individuals to the concentration emitted by our baited traps, rather than from

continuous variations in individual responses. The olfactometric evaluation technique recently developed by our team (Masier et al. 2022) allowed us to characterize individual behaviors on a small scale (4 cm diameter arena): responses to mix1.0 were very heterogeneous between individuals, with each population (including DIN and populations other than those tested here) comprising individuals with opposite responses, whatever the concentration (Masier et al. 2022). In contrast, such a sharp inter-individual heterogeneity was not observed with NH₃ (Masier et al. 2022). This may also partly account for the synergy between mix1.0 and NH₃ in the DIN population reported in the present study.

From our present results, the attractive response to our kairomonal blend appears to be dominated by random exploration and a kinetic attractive response (activating effect without specific orientation; Miller et al. 2009) to kairomone in PRM. The tactic attractive response (source-directed pathway) seems to occur only at very short distances, at least in the DIN population. The degree of directionality of animal foraging movements is highly variable (Pyke 1984). In a context where prey distribution is aggregated and many patches may be empty, predators that optimize prey search are favored over those with low search ability (Pyke 1984). Remote host location may similarly represent an important selective advantage for micropredators. For PRM, the tactic attractant (source-directed pathway) effect of kairomones at medium to large scale may allow the mite to optimize its transits between nests in wild bird colonies. In poultry houses, on the other hand, the ability to develop a tactic attractant response to kairomones may have been counter-selected over generations of PRM under farm conditions. High host density on farms has previously been reported as an important factor in the evolution of livestock parasite life history traits (Mennerat et al. 2010) and host availability is known to significantly affect the evolution of parasite strategies (Crossan et al. 2007). In another study, using an electronic system to trace the entire path of the mite, we measured tortuous paths by PRM averaging 68 cm in 10-min periods in the absence of specific VOCs (Masier et al. 2022). In the present study, we observed that the mite commonly moves 20 to 60 cm away from the release point in one hour. Knowing that the mite stays exclusively inside the poultry house and that the current standard in barn farms in France is 6 to 9 hens per square meter of poultry house, the probability for a mite with such a movement capacity to encounter a host after a few minutes of random exploration in the poultry house is very high. Combined with the negative geotropism of PRM, the random prospecting behavior in the presence of kairomone revealed by medium scale experiments could well contribute to optimizing foraging inside a barn. Our null results from field experiments with traps that have a vertical pillar position similar to that of a hen's foot probably reflect the foraging behaviour of mites maximizing

the chance of contact with the host by taking a tortuous path and climbing on any vertical feature encountered.

Additional studies are needed to integrate these new elements into a more comprehensive framework of understanding, by comparing farm mite populations with wild mite populations (wild avifauna) and by measuring the attractive response with lower concentrations (which will require substantial methodological amendments). Furthermore, other volatile compounds contribute to the formation of the olfactory landscape in poultry houses. In particular, CO₂ is ubiquitous and likely to show considerable concentration variations in the areas frequented by the mite, since it is emitted not only by poultry, but also by micro-organisms growing in the manure and arthropods including red mites. Since it has been shown that it induces, when presented alone to the mite, tactical attractive behavior in PRM in the dark, but on the contrary freezing behavior in full light (Kilpinen 2005), it would be particularly interesting to study the role played by this gas on the behavior of PRM, in interaction with the VOCs emitted by the poultry and NH₃.

Conclusion and Perspectives

For the first time, we clearly showed that an attractant blend for PRM could be made by mixing some VOCs emitted

by hens, a blend that may operate as a kairomone in the host-finding process of some populations of the poultry red mite. To our knowledge, this is the first characterization of a candidate kairomone for a hematophagous mite species. Our results highlight that the chemical interaction between PRM and the host relies on a complex olfactory cue instead of single host volatiles as with most hematophagous insects.

Our study also shows that the attractive response to host-derived olfactory kairomones is not homogeneous in PRM populations that develop on farms. This opens the field for fundamental research on the evolutionary effects of farming activity on host-parasite relationships. It would be interesting to further study the intraspecific variation of olfactory response depending on the concentration of VOCs amongst farms as well as wild birds to unravel the causes of the heterogeneity encountered.

Our results also highlight the need to compare responses between multiple pest populations to develop effective control tools and question the feasibility of developing attract-and-kill systems based on kairomones to control unwinged pests like PRM. Given the small distance for tactic attraction to occur, multiple trapping points would likely be required to achieve trapping that could affect the pest population using our blends (attract and kill strategies). On the other hand, the said blends could provide a way to increase the sensitivity of mite detection for monitoring, currently done mainly with passive traps, by expanding the area represented by each trap

with olfactory bait. Furthermore, targeting attractants other than kairomones, such as aggregation pheromones, could provide a more direct way forward.

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Author Contribution Lise Roy conceived the study, Thomas Auffray and Lise Roy developed the experimental design, Benoît Lapeyre contributed to refine it. Laurent J.M, L. Roy and T. Auffray designed and validated the electric trap. Material preparation, data collection and analysis were performed by Thomas Auffray, Alfonsina Arriaga-Jimenez and Adrien Taudière. The first draft of the manuscript was written by Thomas Auffray and Lise Roy and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The following resources are hosted on the Zenodo Open Science repository:– Raw data provided in two.csv files, corresponding respectively to the data obtained from trap experiments (6-trap lab experiments and field experiments).

– R code written for the statistical analyses carried out on trap experiments.

URL: <https://doi.org/10.5281/zenodo.7356711>

Declarations

Conflict of Interests The authors declare that they have no conflict of interest.

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