LETTER

Birds exploit herbivore-induced plant volatiles to locate herbivorous prey

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Abstract

Arthropod herbivory induces plant volatiles that can be used by natural enemies of the herbivores to find their prey. This has been studied mainly for arthropods that prey upon or parasitise herbivorous arthropods but rarely for insectivorous birds, one of the main groups of predators of herbivorous insects such as lepidopteran larvae. Here, we show that great tits (*Parus major*) discriminate between caterpillar-infested and uninfested trees. Birds were attracted to infested trees, even when they could not see the larvae or their feeding damage. We furthermore show that infested and uninfested trees differ in volatile emissions and visual characteristics. Finally, we show, for the first time, that birds smell which tree is infested with their prey based on differences in volatile profiles emitted by infested and uninfested trees. Volatiles emitted by plants in response to herbivory by lepidopteran larvae thus not only attract predatory insects but also vertebrate predators.

Keywords

Apple trees, avian olfaction, foraging, induced indirect plant defence, insect herbivores, insectivorous birds, multitrophic interactions, *Parus major*.

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INTRODUCTION

Among the most exciting interspecific relationships mediated by chemical cues are multitrophic interactions involving plants, herbivorous arthropods and carnivorous arthropods. When a plant is attacked by herbivorous arthropods, it induces a defence response. The metabolites that the plant produces as a defence may directly affect the performance of the herbivorous arthropod (*induced direct defence*) by, e.g. inhibiting feeding behaviour of insects, decreasing digestibility or intoxicating the insect (Schoonhoven *et al.* 2005). Furthermore, it has been proposed that the volatiles that plants emit upon attack by herbivorous arthropods have an indirect defence function by attracting carnivorous enemies of the herbivores (*induced indirect defense*, Dicke *et al.* 1990a; Turlings *et al.* 1990; Turlings & Tumlinson 1992; Vet & Dicke 1992). In doing so, plants may reduce the damage by the herbivore, and thus can enhance their fitness (van Loon *et al.* 2000; Fritzsche Hoballah & Turlings 2001; Schuman *et al.* 2012).

The phenomenon of herbivore-induced emission of volatile organic compounds by plants has mainly been studied considering insect enemies of the herbivores (see Mumm & Dicke 2010; Dicke & Baldwin 2010 for reviews). However, many bird species, such as the great tit, *Parus major*, are voracious predators of herbivorous insects such as lepidopteran larvae, including the winter moth (*Operophtera brumata*, Lepidoptera, Geometridae). Because the nestling

period of the great tit coincides with the peak occurrence of winter moth larvae, birds can greatly reduce the number of lepidopteran larvae feeding on trees (Mols & Visser 2002). Predation of winter moths by great tits has been found to decrease herbivore damage to trees (Mols & Visser 2002; Van Bael *et al.* 2003; Mäntylä *et al.* 2011). This leads to increased growth and reduced mortality of the trees (Marquis & Whelan 1994; Sipura 1999; Mäntylä *et al.* 2011). Therefore, plants that are infested by herbivorous insects could benefit from the attraction of insectivorous birds. Furthermore, insectivorous birds could also benefit from the use of plant cues that enhance their chances to find their herbivorous prey. The prey itself usually emits low amounts of cues thereby reducing detection by predators (Rowland *et al.* 2008), whereas information emitted by the plant may be much easier to detect due to the considerably larger biomass of plants compared to herbivores (Vet & Dicke 1992). Previous evidence suggests that birds are attracted to trees infested by lepidopteran larvae, without the need to see larvae or their damage on leaves (Mäntylä *et al.* 2004, 2008a,b), but the mechanism underlying the attraction remains unknown. Here, we present experiments aimed to elucidate whether birds are attracted to trees infested by herbivorous prey and to explore the mechanism underlying such attraction in the system: great tits – winter moths – apple trees.

To examine whether birds are attracted to trees infested by lepidopteran larvae, we performed a two-choice experiment in an aviary (Fig. 1) containing two types of apple trees, one control and one

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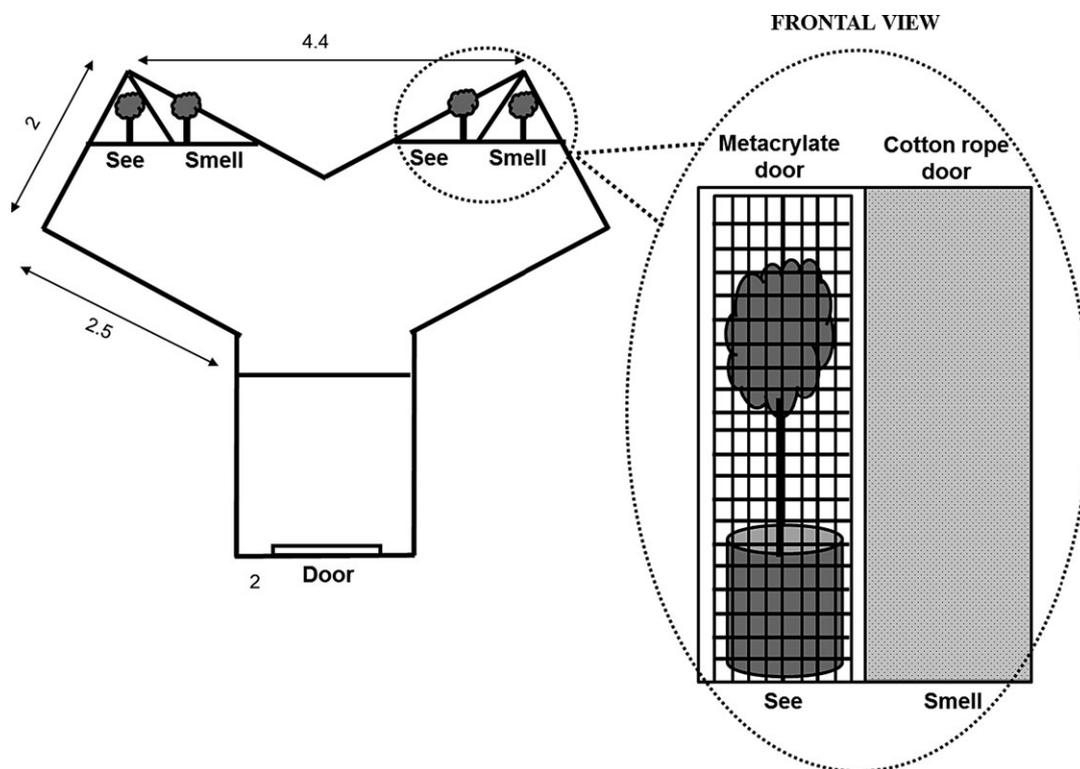
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Figure 1 Schematic representation of the aviaries used for the experiments. Numbers indicate size in metres. This experiment was aimed to disentangle whether birds detected the chemical or the visual cues of infested trees. Each pair of apple trees was located inside a compartment with two compartments. The door of one compartment was made of methacrylate to allow the bird to see the tree, but not to smell the tree. The door of the other compartment was made with cotton material to allow the bird to smell the tree, but not to see it. In experiments aimed to examined the attraction of birds to infested trees, the same aviaries were used but without compartments. In these experiments, one tree was located in the same place that each compartment separated from the rest of the aviary by a mesh.

experimental tree. We investigated the first visit and the proportion of visits by the birds to the tree that was experimentally infested with winter moth larvae. We tested whether great tits preferred (1) trees infested with larvae, (2) trees containing damaged leaves, from which larvae had been removed or (3) trees infested by larvae, from which both larvae and the damaged part of each leaf had been removed. If birds are able to use larva-induced tree volatiles we expected birds to prefer the tree infested by lepidopteran larvae, even when larvae or the damaged leaves had been removed before the choice experiment.

Next, we analysed the mechanism responsible for the preference of great tits for infested trees. We examined whether great tits were attracted to (1) chemical cues, (2) visual cues, (3) chemical & visual cues of apple trees infested with lepidopteran larvae, from which damaged parts of leaves and the larvae themselves had been removed just prior to the experiment. To further explore the potential cues used by the birds, we quantified the chemical and visual differences between infested and uninfested trees. We expected that infested trees differed from the uninfested trees in the visual and chemical cues that they emitted. Predatory arthropods are known to discriminate between infested and uninfested trees based on the chemical cues that plants emit (Schoonhoven *et al.* 2005). Although birds are traditionally considered to primarily use vision, recent evidence suggests that olfaction may be used more often than previously thought, also in foraging contexts (e.g. Nevitt 2011). Therefore, we also expect birds to, at least partly, rely on chemical cues to discriminate between infested and uninfested trees.

MATERIALS AND METHODS

Insect species

In November 2006 and 2007, winter moth females (*Operopthera brumata* L.) were captured in several deciduous forests to the west of Arnhem (05°48′ E, 51°59′ N), the Netherlands. Females were kept individually in 50 mL falcon tubes (30 mm O.D., 115 mm length) to lay their eggs. Clutches were kept in petri dishes in outdoor conditions until March, when eggs were transferred to climate cabinets (SANYO Incubator MIR-553) and maintained at 12 °C. Fresh young leaves of peach and apple trees were provided to the containers to ensure that newly hatched larvae would have food. Larvae were reared on these leaves until they reached the fifth larval instar (L5).

Tree species

From the beginning of April 2007 and 2008 we placed thirty-five 1.5 m tall apple trees, *Malus silvestris* Miller (variety De Costa), planted in 40 L pots inside a greenhouse for two weeks before the development of leaves. After leaf development, trees were moved outdoors to habituate to experimental conditions. We separated control and experimental trees several metres apart (minimum 10 m) to avoid interactions between them.

Three days before the experiment, we individually placed 30 winter moth (*O. brumata*) larvae (L5) inside clip-cages (Ø = 250 mm) on each tree assigned the 'infestation' treatment. In this way, larvae

could eat the leaf but could not move from one leaf to another. We used 30 larvae because we wanted to mimic a natural situation, where birds can find larvae in some but not in all tree leaves. Uninfested trees were maintained without larvae.

Bird species

We used naïve captive adult great tits, *Parus major*, housed individually in $0.9 \times 0.4 \times 0.5$ m cages. Birds were 1 year old and all of them were hand-reared since they had been 10 days old; therefore, they did not have any previous experience in foraging among trees.

Before the experiments, all birds were habituated to the aviary by releasing them once during one hour inside the aviary without apple trees. In all experiments, we removed the food from the cages that housed the experimental birds one hour before each trial to ensure that the birds were motivated to search for larvae during the experiment. After the trial, the bird was captured with a net and returned to its cage. Birds did not show signals of stress during the trials and when they were returned to their cages they immediately resumed their normal behaviour. All experiments were carried out under licensc of the Animal Experimental Committee of the KNAW (DEC protocol no CTE 07.01).

We used thirty-eight adult great tits to test whether birds were attracted to trees infested with lepidopteran larvae, and thirty-five other birds to examine the mechanism underlying the discrimination between infested and uninfested trees. A repeated measure design was used in both experiments. All birds were tested in the three treatments in a randomised order. Only one trial was conducted per bird per day, and there was at least one day without testing between trials. Before these experiments, birds were trained five times to acclimatise them to the aviary and to allow them to find larvae in the apple trees. During habituation trials, the mesh was partially removed (in the experiment to assess the attraction to infested trees) or the door opened (in the experiment to unravel the mechanism underlying the attraction to infested trees) to allow birds to have access to both trees that were equally infested with larvae in these trials. To maintain the birds' interest to search for larvae during the trials, between each trial of the experiment, we performed one habituation trial with each bird to allow it to eat larvae from trees at both locations of the trees within the aviary simultaneously.

Experimental design

Experiments were performed in late April and early May in 2007 and 2008 in two outdoor Y-shaped aviaries built with mesh screens (mesh size 1.3 cm) (Fig. 1). Each branch of the aviary was $2.5 \times 2 \times 2$ m (l × w × h). The central branch was closed 72 cm near the intersection with the other two branches. The aviary contained three perches, one near each tree and the third in the middle of the aviary.

Experiment 1: attraction to infested trees

In this experiment, two apple trees were placed at the end of the branches of the aviary, separated 4.40 m from each other. One of the trees was uninfested and the other tree infested. The infested tree had one of the following treatments: (1) 'Caterpillar' (infested tree with 30 lepidopteran larvae and damaged leaves), (2) 'Damaged leaves' (infested tree with damaged leaves from which the larvae

had been removed) or (3) 'Previously infested' (infested tree from which larvae and damaged parts of leaves had been removed). The larvae and damaged parts of leaves were removed just before the trials. We cut the damaged parts of leaves in the 'previously infested' treatment. We cut the part of the leaf that had been in contact with the larvae to remove not only the visual damage but also any chemical compound left by larvae such as faeces. We removed the clip-cage containing the larvae by cutting the part of the leaf where the clip-cage was located (about half a leaf). We also cut a similar part of the same number of leaves in the uninfested trees and in the infested trees in the other treatments. Trees were covered with protective mesh, to prevent birds from eating the larvae in the 'caterpillar' treatment. We used 18 different pairs of trees.

Experiment 2: mechanism underlying the attraction to infested trees

Infested and uninfested trees (see above) were obtained as previously described. We removed larvae and damaged leaves from infested trees and removed a similar number of leaves in control, uninfested trees. Therefore, infested trees were similar to those of the 'previously infested' treatment in the former experiment. In this experiment, two apple trees where placed at the end of each branch of the aviary and we thus had four apple trees in the aviary (Fig. 1). Each pair of trees was located in a compartment with two parts. One of the parts of the compartment contained a methacrylate door, and the tree could be seen but not smelled. The other part of the compartment contained a cloth (cotton) door, so the tree could be smelled but not seen by the birds. One of the pairs of trees was control and the other one experimental. In the control pair of trees, trees were always uninfested. The experimental pair of trees could have one of the following treatments: (1) 'chemical', (2) 'visual', (3) 'chemical and visual'. In the 'chemical' treatment, the tree that could be seen was uninfested and the tree that could be smelled was infested. In the 'visual' treatment, the tree that could be seen was infested and the tree that could be smelled was uninfested. In the 'chemical and visual' treatment, both trees were infested, and therefore, birds could smell and see an infested tree. We used 12 different groups of 4 trees.

EXPERIMENTAL PROCEDURE

Trials were performed between 09:00 and 17:00 and under sunny and warm conditions (mean ± SE temperature = 20 ± 1 °C) to avoid variation in the emission of volatiles due to differences in ambient conditions such as temperature (Vallat *et al.* 2005). On each test day, a new control and experimental tree or pair of trees was placed in each aviary. We randomised the place of the trees (right or left) as well as the aviary (number one or number two) among trials. Trees were tested with several birds (mean and median = 6 birds, from two to a maximum of 7 birds). We recorded the behaviour of birds during 30 min using a video camera. An observer, blind to the treatments, analysed the video tapes and recorded the first tree inspected by the bird and the number of visits to each tree during 30 min in the two experiments. We calculated the proportion of visits to the control and experimental tree.

We analysed the first choice as well as proportion of visits to the experimental tree by using generalised linear mixed models fit

by the Laplace approximation, with these variables following a binomial distribution with logit function. The individual as well as the tree pair were included in the model as random factors. Treatment, tree location (left or right), aviary location (left or right) and order of trial were included in the model as fixed factors, when relevant. Day, hour, hour^2 and temperature were also included in the initial models but were removed when they were not significant. Treatment effect was calculated by comparing the models with and without the treatment with ANOVA. Analyses were performed with the Statistical package R 2.15.1 (R Development Core Team 2012). Cases where birds did not visit any tree where excluded from the analysis (two cases in the experiment to assess the attraction to infested trees, and 23 cases in the experiment to disentangle the mechanism responsible for the attraction to infested trees).

Chemical analysis through GC-MS

To further elucidate the mechanism underlying the attraction to infested trees, we analysed the volatile organic compounds emitted by 32 individual trees (16 infested and 16 uninfested) right after the behavioural tests, between 16:30 and 19:00 during eight experimental days. We collected the volatiles of a subset of the total number of trees that were used in the trials with birds (two infested and two uninfested trees each day). We selected one branch of each tree with a similar number of leaves among trees and introduced 20 cm of the branch into a 25 × 38 cm polyethylene oven bag (Toppits®, Melitta, Lokeren, Belgium). To remove volatile organic compounds, the bags had been heated for 4 h at 120 °C before use (Stewart-Jones & Poppy 2006). Bags were fastened to the bark of the branch with tape and one of the two outermost bag corners was cut to allow the placement of a tube containing a steel trap filled with 150 mg Tenax TA and 150 mg Carbopack B (Markes International Limited, Llantrisant, UK). The trap was connected to a vacuum pump. Collection flow rates were set to 200 mL/min. After 2 h, the traps were removed and capped till analysis. We also measured two background VOC profiles from empty bags on two of the days. The values of compounds in these background samples were subtracted from values in the tree samples. Traps were stored at 4 °C for 10–11 weeks until analysis. Volatiles were desorbed from the traps using an automated thermodesorption unit (model Unity, Markes, Llantrisant, UK) at 200 °C for 12 min (He flow 30 mL/min) and focused on a cold Tenax trap (−10 °C). After 1 min of dry purging, trapped volatiles were introduced into the GC-MS (model Trace, ThermoFinnigan, Austin, Texas) by heating the cold trap for 3 min to 270 °C. Split ratio was set to 1 : 4 and the column used was a 30 × 0.32 mm ID RTX-5 Silms, film thickness 0.33 μm. Temperature program: from 40 °C to 95 °C at 3 °C/min. then to 165 °C at 2 °C/min, and finally to 250 °C at 15 °C/min. The volatiles were detected by the MS operating at 70 eV in EI mode. Mass spectra were acquired in full scan mode (33–300 amu. 0.4 scan/sec). Compounds were identified by their mass spectra using deconvolution software [AMDIS; NIST (International Institute of Standards and Technology, MD, USA) and US DOD (Department of Defense, DC, USA)] in combination with Nist 98 and Wiley seventh edition spectral libraries and by comparing their linear retention indices. In addition, mass spectra and/or linear retention indices of chromatographic peaks were compared with values reported in the literature.

Additional confirmation for compound identification was obtained by interpolating retention indices of homologous series, or by comparing analytical data with those of reference substances. The integrated signals generated by the AMDIS software from the MS-chromatograms were used for comparison between the treatments. Peak areas in each sample were divided by the total volume in ml that was sampled over the trap, to correct for small differences in flow rates over individual traps.

We used an in-house written routine for Orthogonal PLS-DA for MATLAB (Bylesjö *et al.* 2006), R2010a (Mathworks, Natick MA) to generate a model to describe the general effect of treatment, by contrasting the chemical profiles emitted by trees in the control group against those emitted by trees infested with lepidopteran larvae. The data were log-transformed and the average emission of all trees per day was removed from the data, to remove day-to-day variation caused by non-experimental factors. We then determined the two latent variables for the model, by single cross-validation (Westerhuis *et al.* 2008). We subsequently quantified the significance of the model result by calculating the F-ratio of the obtained class predictions against those from a permutation analysis, where factor 'time' was left intact but the 'treatment' factor was permuted (Anderson & Ter Braak 2003). These showed that 1000 permuted models all discriminated both treatments less well than that on the original data. We identified the volatiles with largest OPLS-DA weights as significant for the treatment. This analysis did not identify a significant change in the chemistry that underlies this difference during the 14 days of the experiment.

Colouration measurements

We also collected five leaves from infested ($n = 16$) and uninfested trees ($n = 15$) used in the behavioural trials and measured colouration. Colour measurements were performed by using a USB-2000 spectrophotometer with a DH-2000 deuterium–halogen light source (both Avantes, Eerbeek, the Netherlands). During the measurement of each leaf, we took three replicate readings and obtained the reflectance spectra of each measurement. We calculated the total reflectance of leaves between 300 and 700 nm, which include the spectral range visible to birds (320–700 nm, Cuthill 2006). We also calculated the UV reflectance (between 300 and 400 nm) and human visible reflectance (400–700 nm). Leaf colour measurements were highly repeatable within leaves (repeatability Total reflectance = 0.999; $F_{154, 310} = 6.46$; repeatability UV reflectance = 0.999; $F_{154, 310} = 3.30$; repeatability human-visible reflectance = 0.999; $F_{154, 310} = 6.66$) and within trees (repeatability Total reflectance = 0.996; $F_{30, 434} = 4.48$; repeatability UV reflectance= 0.998; $F_{30, 434} = 2.35$; repeatability human-visible reflectance = 0.996; $F_{30, 434} = 4.61$). Differences between infested and uninfested trees in the reflectance of leaves were analysed using GLM with STATISTICA, controlling for the day as a random factor.

RESULTS

Significantly more birds paid the first visit to the infested tree than to the control uninfested tree (Fig. 2a). This preference for the infested tree was found in all treatments (no differences in strength of preference among treatments ($\chi^2 = 0.36$, d.f. = 2, $P = 0.83$; Fig. 2a; significance levels for when the infested tree contained lar-
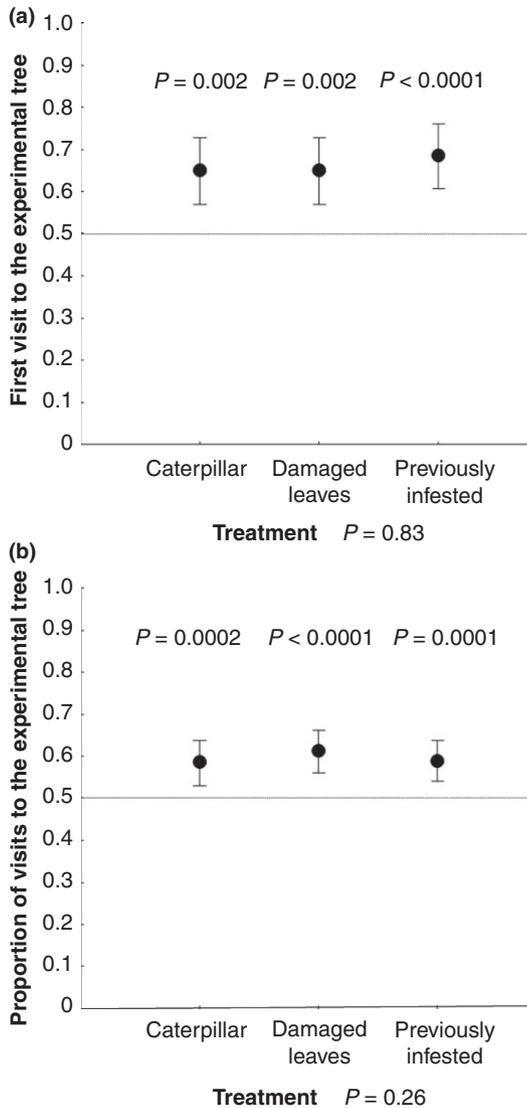
Figure 2 Mean ± SE of (a), Number of birds that paid the first visit and (b), Proportion of visits to the experimental infested tree by great tits, *Parus major* (n = 38), when released in an aviary with two apple trees: one control (uninfested) and one experimental (infested). The experimental, caterpillar-infested tree had one of the treatments: (1) tree with *Operopthera brumata* caterpillars feeding on the leaves ('caterpillar'), (2) tree with leaves damaged by caterpillars that were removed before testing ('damaged leaves'), (3) tree previously damaged by caterpillars but damaged parts and caterpillars were removed before testing ('previously infested').

Figure 3 Mean ± SE of (a), Number of birds that paid the first visit and (b), Proportion of visits to the experimental infested tree by great tits, *Parus major* (n = 35), when released in an aviary with two pairs of apple trees, one control (uninfested) and one experimental (infested). The experimental tree pair had one of the following treatments: (1) Chemical cues, (2) Visual cues and (3) Chemical and Visual cues (c.f. 'Previously infested' in Fig. 2) released by apple trees under *Operopthera brumata* caterpillar herbivory. Caterpillars and damaged leaves were removed before the experiment.

vae and damaged leaves: Z = 3.14, P = 0.002; only damaged leaves without larvae: Z = 3.08, P = 0.002; neither larvae or damaged leaves ('previously damaged'): Z = 3.40, P = 0.0007). The birds also visited the infested tree more frequently than the uninfested tree during the 30 min observation (Fig. 2b). Again, this was similar for all three treatments (χ^2 = 2.67, d.f. = 2, P = 0.26; 'caterpillar': Z = 3.77, P = 0.0001, 'damaged': Z = 4.01, P < 0.0001, and 'previously damaged': Z = 3.80, P = 0.0001), and thus the birds were attracted even when they could not see the caterpillars or their feeding damage.

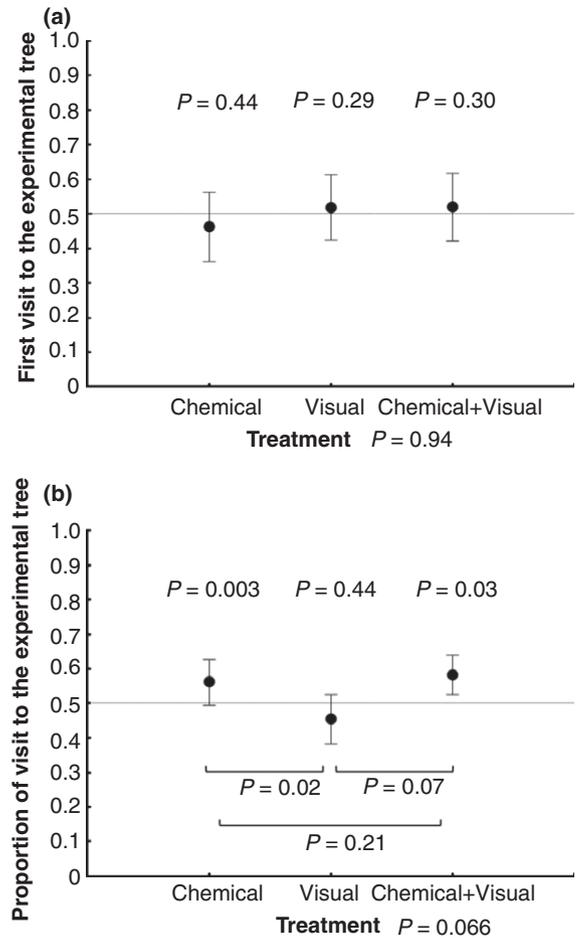Furthermore, in tests addressing the cues used by the birds, their preference for infested trees, measured as the proportion of visits, was only exhibited when the only cues available were chemical cues (Fig. 3b; Z = 2.99, P = 0.003), but not when there were only visual cues (Z = 0.77, P = 0.44; difference between chemical and visual cues only: χ^2 = 5.54, d.f. = 1, P = 0.02). In contrast to the first experiment, in this experiment the first choice did not differ between infested and uninfested trees (P > 0.29 in all cases) or between treatments (χ^2 = 0.12, d.f. = 2, P = 0.94, Fig. 3a).

Leaves from infested trees differed visually from leaves from uninfested trees (Fig. 4), with infested trees having a lower leaf reflectance than uninfested trees both in the visual ($F_{1,22}$ = 9.32, P = 0.006) and UV spectral range ($F_{1,22}$ = 4.80, P = 0.04). Trees infested by lepidopteran larvae also differed from uninfested trees in their volatile profiles (see Table S1), as demonstrated by a validated Partial Least Squares-Discriminant Analysis. They emitted more α-farnesene and dodecanal, while they emitted less 1,2,4 trimethyl benzene, 1-octen-3-ol, methoxy phenyl oxime, 1-nonene, and 3-octanol compared to control uninfested trees (Fig. 5).
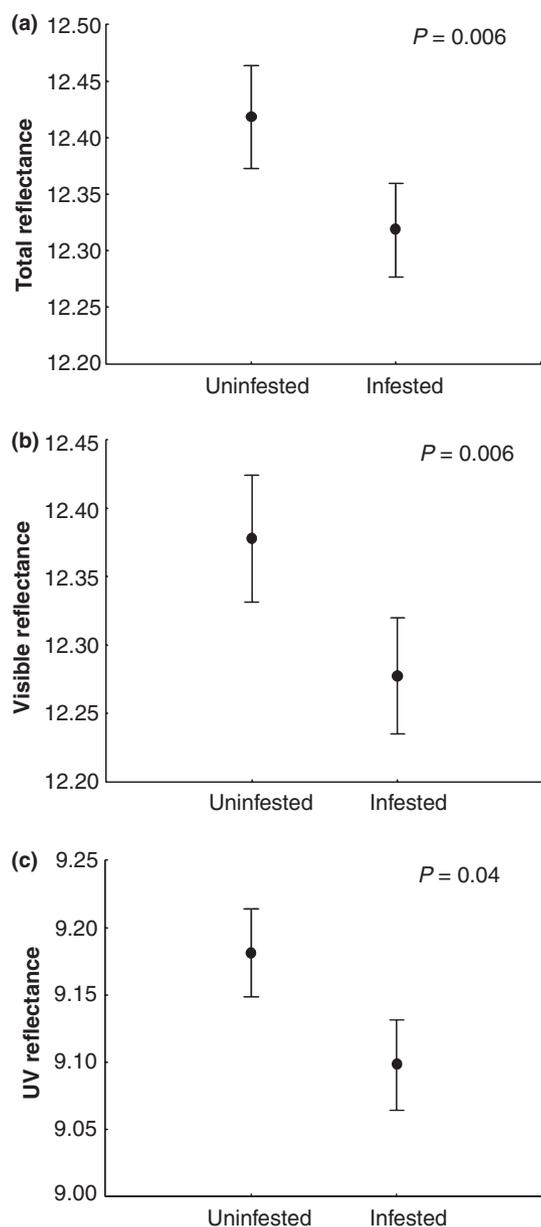
Figure 4 Spectral analysis of leave colouration. Log-transformed mean ± SE of (a), Total Reflectance (300–700 nm), (b), human-Visible Reflectance (400–700 nm), and (c), UV Reflectance (300–400 nm) of control uninfested apple trees and apples trees infested with *Operopthera brumata* caterpillars.

Figure 5 Chemical analysis. Log-transformed mean ± SE relative emission rates (peak area per ml volume sampled) of chemical compounds for which emission rates differed, accordingly to PLS-DA, between control uninfested apple trees and apples trees infested with *Operopthera brumata* caterpillars.

DISCUSSION

Our results show that great tits exploit herbivore-induced plant volatiles to locate herbivorous prey. The birds were attracted to trees infested by lepidopteran larvae, even when we had removed the larvae and their feeding damage just before the experiment. This allowed us to exclude the option that birds could see the larvae or their feeding damage (Fig. 2). Thus, the preference for infested trees was not due to the visible damage resulting from larval feeding on the leaves, or by chemical cues associated with the larvae such as silk or faeces. A potential explanation for this is that these cues may not accurately signal the current availability of prey in a tree. For example, the presence of damaged leaves on a tree may cause
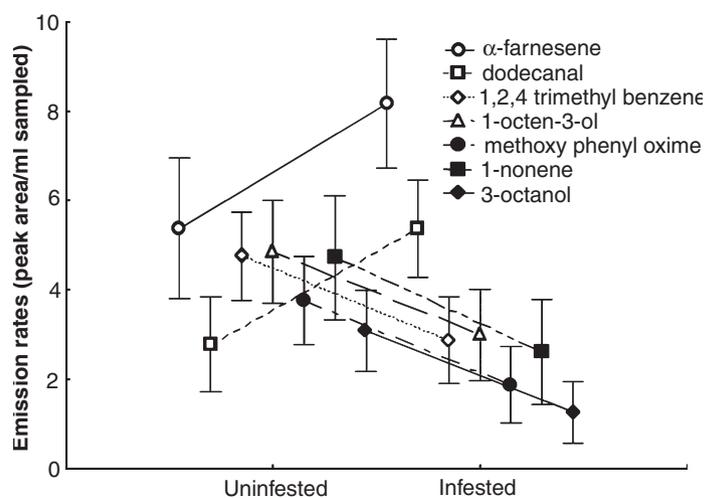
an overestimation of the presence of prey because the damaged leaves remain much longer on the tree than the larvae, which could have been preyed upon or could have left the tree for pupation.

Our results show that birds can discriminate between infested and uninfested trees based on the induced response of the tree. Our results are in accordance with previous studies that recorded the attraction of passerine birds to infested trees (Mäntylä *et al.* 2008a,b). In these previous studies, however, the cues responsible for the attraction to infested trees were not separated and it was therefore not possible to conclude whether visual cues, chemical cues or both were responsible for this attraction (Mäntylä *et al.* 2008a,b). In contrast, we offered birds chemical or visual cues alone or in combination. By doing so, we have shown that bird attraction to infested trees was mainly mediated by chemical cues from the tree, i.e. bird preference for infested trees was still exhibited when the only cues available were plant volatiles (Fig. 3), but not when there were only visual cues. This demonstrates that birds were attracted by the induced emission of volatiles by the tree rather than by the larvae themselves, the visual damage caused by the larvae or the visual cues of undamaged leaves from the infested tree. Similar findings have been reported in previous studies with predatory and parasitoid arthropods (Dicke *et al.* 1990a; Turlings *et al.* 1990; Turlings & Tumlinson 1992; Vet & Dicke 1992; Mumm & Dicke 2010) but never for vertebrate predators.

Infested trees differed visually from uninfested trees (Fig. 4), with infested trees having a lower leaf reflectance than uninfested trees both in the visual and the UV spectral range. Therefore, the colouration of leaves could be a cue to ascertain the level of herbivory of trees. However, visual cues may not be a reliable cue because the reflectance of leaves may be related to other factors affecting trees rather than herbivory, such as sunlight exposure (Mäntylä *et al.* 2008a).

Trees infested by lepidopteran larvae also differed from uninfested trees in their volatile profiles (see Table S1), emitting, among others, more α-farnesene compared to control uninfested trees (Fig. 5). The sesquiterpenoid α-farnesene is present both in the headspace of apple leaves (Takabayashi *et al.* 1991) and apple fruits

(Boeve *et al.* 1996; Landolt *et al.* 2000), and, at least for fruits, it is involved in the attraction of both herbivorous and predatory insects (Boeve *et al.* 1996; Landolt *et al.* 2000). We show that this compound is also present in the headspace of apple trees that are infested with winter moth larvae and, thus that the birds can potentially make use of it when locating infested trees. However, further research is needed to establish which compound or mixture of compounds (Bruce & Pickett 2011) is responsible for bird attraction, as well as to understand how differences in emission rates between infested and uninfested trees modulate bird choice behaviour.

The observation that birds use the volatiles from infested trees to find their prey is in line with other studies on avian olfaction in foraging, and indicates that the importance of olfaction in avian life history may be greater than was previously thought. Phytoplankton releases chemicals to the seawater in response to zooplankton grazing that is converted into dimethyl sulphide (DMS) that is emitted to the air (Pohnert *et al.* 2007). Hence, DMS signals areas of high productivity in the ocean (Nevitt 2011) and several species of Procellariiformes seabirds (Nevitt *et al.* 1995; Nevitt 2011) and penguins (Amo *et al.* 2013) use DMS to locate these productive areas (Nevitt 2011). Indeed, to use chemical cues during foraging seems to be an ancient trait in birds (e.g. Kiwis *Apteryx australis* (Cunningham *et al.* 2009); *Cathartes* vultures (Gomez *et al.* 1994)), and it persists in several modern lineages (Procellariiforms (Nevitt *et al.* 1995); chinstrap penguins (Amo *et al.* 2013); zebra finches (Kelly & Marples 2004); and domestic chicken (Marples & Roper 1996)).

The ability to detect the chemical cues emitted by infested trees may especially be important for insectivorous birds such as great tits or blue tits that feed nestlings on lepidopteran larvae, a resource that is variable in space and time (Perrins 1991) and is abundant only for a very short period (Naef-Daenzer *et al.* 2000). Therefore, the benefits for the birds of using induced volatiles from trees are obvious in terms of increased fitness. From the infested tree's point of view, the attraction of insectivorous birds can greatly reduce the number of feeding larvae (Mols & Visser 2002), may be beneficial in terms of decreased leaf damage and plant mortality (Mäntylä *et al.* 2011), and therefore, may have a positive impact on fitness. Therefore, our results add to the abundant literature showing that induced plant volatiles attract predators (reviewed by Vet & Dicke 1992; Mumm & Dicke 2010), this time for vertebrate predators. This novel evidence of the ability of insectivorous birds to use chemical cues of infested plants to locate herbivorous prey is exciting because of the high predation rates of birds compared to those of predatory arthropods. This further supports the incentive for plant breeding to enhance the genetic trait underlying the induced volatile emission from plants that are being attacked by insects (Dicke *et al.* 1990b) and in such a way maximise the impact of insectivorous birds in the biological control of insect pests.

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STATEMENT OF AUTHORSHIP

L.A., M.E.V. & M.D. planned the experiments and wrote the manuscript. L.A. carried out the experiments and took visual and chemical measurements of trees. N.M.v.D. advised on volatile sampling and chemical compound analysis. L.A. performed the statistical analysis of data, except the PLS-DA which was carried out by J.J.J.

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