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ORIGINAL ARTICLE

The attraction of insectivorous tit species to herbivore-damaged Scots pines

Elina Mäntylä[1] · Sven Kleier[1] · Silke Kipper[2,3] · Monika Hilker[1]

Abstract Insectivorous birds are attracted to insect-damaged deciduous trees by plant cues. However, it is unknown whether birds can locate insects on coniferous trees by plant cues induced by insect feeding activity. Feeding damage may induce plant chemical changes even in parts of a tree where no actual infestation is present. In a laboratory set-up, we studied the behavioural responses of Great Tits (*Parus major*) and Blue Tits (*Cyanistes caeruleus*) to Scots pine *(Pinus sylvestris)* that had been systemically herbivore-induced by pine sawfly larvae (*Diprion pini)*. When birds were simultaneously offered an untreated control Scots pine branch and a systemically herbivore-induced test branch (i.e. a branch without actually feeding larvae) in a study booth, they visited the systemically herbivore-induced test branch first significantly more often than the control branch. In order to elucidate whether this discrimination was based on visual and/or olfactory plant cues, we compared the light reflectance and odour of test and control branches in a separate experiment. The control branches tended to show higher light reflectance throughout the avian vision range of 300–700 nm than the test branches, but the discrimination threshold model suggests that birds are not able to detect this difference. The

systemically herbivore-induced branches emitted the same 29 volatile compounds as control branches, but 21 of them were released in greater quantities by the test branches. Our study shows that herbivore-induced Scots pine branches emit olfactory cues that may allow the birds to discriminate between uninfested and insect-infested trees, while the role of visual, systemically induced plant cues is less obvious and needs further investigation.

Keywords *Cyanistes caeruleus* · Herbivory · Olfaction · *Parus major* · Systemic induction · Vision · Volatile organic compounds

Zusammenfassung

Insektivore Meisen-Arten werden von fraßgeschädigten Waldkiefern angelockt.

Durch Insekten geschädigte Laubbäume produzieren Signale, die für insektivore Vögel attraktiv sind. Ob Vögel auch Insekten an Nadelbäumen durch Signale, die die Pflanze aufgrund der Fraßaktivität der Insekten produziert, wahrnehmen können, ist dagegen unbekannt. Fraßschäden können systemisch chemische Veränderungen selbst in Teilen der Pflanze induzieren, die gar nicht von Schädlingen befallen sind. In einem Experiment unter kontrollierten Bedingungen untersuchten wir die Verhaltensantworten von Kohlmeisen (*Parus major*) und Blaumeisen (*Cyanistes caeruleus*) auf Waldkiefern (*Pinus sylvestris*), die zuvor systemisch durch Befall von Larven der Kiefernbuschhornblattwespe (*Diprion pini*) induziert wurden. Wenn Vögel in einer Testbox zwischen einem unbehandelten Kontrollast einer Kiefer und einem systemisch fraßinduzierten Ast wählen konnten, besuchten sie signifikant häufiger zuerst den fraßinduzierten Ast. Um

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zu untersuchen, ob diese Unterscheidung zwischen ungeschädigtem und fraßinduziertem Kiefernast auf visuellen und/oder olfaktorischen Pflanzensignalen basierte, verglichen wir die Lichtreflexion und den Geruch von Kontroll- und induzierten Ästen. Die Kontrolläste hatten tendenziell höhere Lichtreflexionswerte im kompletten Bereich des für Vögel sichtbaren Lichtspektrums (300–700 nm). Ein Diskriminierungsschwellenmodell sagte jedoch vorher, dass diese Unterschiede zu gering sind, um von den Vögeln wahrgenommen zu werden. Die systemisch fraßinduzierten Äste verströmten die gleichen 29 Duftkomponenten wie die Kontrolläste; 21 davon wurden jedoch von den fraßinduzierten Ästen in höheren Mengen abgegeben. Unsere Studie zeigt, dass systemisch fraßinduzierte Kiefernäste olfaktorische Signale produzieren, die es Vögeln ermöglichen, zwischen unbefallenen und von Insekten befallenen Bäumen zu diskriminieren. Die Rolle von visuellen, systemisch induzierten Signalen ist dagegen nicht eindeutig geklärt und bedarf weiterer Untersuchungen.

Introduction

Infestation of plants by herbivorous invertebrates is well known to induce numerous defensive plant responses (Walling 2000; Howe and Jander 2008). Among them, induction of herbivore-induced plant volatiles (HIPVs) has been paid much attention. Feeding damage of plants by herbivores not only induces changes in the emission of plant volatiles locally, but also systemically so that undamaged parts of herbivore-infested plants also release HIPVs (reviewed by Orians 2005; Wu and Baldwin 2009). Numerous studies have shown that HIPVs can attract predatory and parasitic arthropods to the entire herbivore-infested plant (reviewed by Karban and Baldwin 1997; Holopainen 2004; D'Alessandro and Turlings 2006; Dicke 2009).

Herbivore-induced plant changes are not only exploited by arthropod enemies of herbivorous insects and mites, but also insectivorous birds can use these cues to locate herbivorous larvae. For example, birds were more attracted to herbivore-damaged birches (*Betula* spp.) than to undamaged controls, although they saw neither the larvae [e.g. autumnal moths (*Epirrita autumnata*)] nor the damaged leaves (Mäntylä et al. 2004, 2008a, b, 2014; but see Koski et al. 2015). Similarly, herbivore-damaged crab apple trees (*Malus sylvestris*) attracted insectivorous birds by their HIPVs (Amo et al. 2013). Naïve birds did not recognise these herbivore-damaged apple trees but 5 h practice was enough to train them (Amo et al. 2016).

The plant cues that inform birds about the presence of herbivorous larvae may be visual or olfactory. While at the actual local site of herbivory the visual cues of the herbivore itself and feeding holes may be attractive to birds, herbivore-induced changes of plant visual cues may also occur systemically at undamaged parts of an infested plant. These changes in leaf colour and light reflectance may be due to systemically feeding-induced changes in photosynthetic activity (Zangerl et al. 2002; Peñuelas et al. 2004; Nykänen and Koricheva 2004; Nabity et al. 2009; Pinkard et al. 2011).

The vision of birds covers a wider spectrum (four cone cell types; 300–700 nm) than that of humans (three cone cell types; 400–700 nm), and coloured oil droplets over the cone cells enable birds to see more hues than humans (Cuthill 2006; Jones et al. 2007; Lind et al. 2014). The finding that undamaged silver birches (*Betula pendula*) reflect more light throughout the avian-visible spectrum than herbivore-damaged birches supports a potential role of vision for the recognition of such damage (Mäntylä et al. 2008b). Additionally, the net photosynthesis rate was lower in herbivore-damaged mountain birches (*Betula pubescens* ssp. *czerepanovii*) than in undamaged trees (Mäntylä et al. 2008a). On the other hand, Amo et al. (2013) did not find support for the role of avian vision in recognising herbivore-damaged trees, but showed that captive birds located herbivore-damaged trees by olfactory cues.

The olfactory ability of most birds, including passerines, was long thought to be negligible (Roper 1999). However, Steiger et al. (2008) found birds to have an underappreciated olfactory sense. Recent studies have shown that passerines use olfactory cues in many situations, including foraging, kin recognition, aromatising nests, navigation and recognising predators (Wallraff et al. 1995; Petit et al. 2002; Mennerat et al. 2005; Amo et al. 2008, 2011, 2013; Gwinner and Berger 2008; Holland et al. 2009; Krause et al. 2012, 2014; Gagliardo 2013).

Since several meta-analyses have shown that plants benefit from the presence of insectivorous birds (Van Bael et al. 2008; Mooney et al. 2010; Mäntylä et al. 2011), a plant's ability to inform birds about the presence of insect prey by herbivore-induced visual and/or olfactory cues is expected to be widespread among plant species. While deciduous trees are known to attract birds by herbivore-induced plant changes, it is unknown whether feeding-damaged coniferous trees also display traits that are exploited by foraging birds for location of prey.

Both deciduous and coniferous trees may change their photosynthetic activity in response to herbivory (Vanderklein and Reich 2000; Copolovici et al. 2011; Eyles et al. 2011; Bansal et al. 2013) and thus change the hue of their leaves or needles. Furthermore, both deciduous and coniferous trees release huge amounts of volatiles in response to herbivory (Blande et al. 2009; Holopainen

2012). The herbivore-induced odour of coniferous trees is especially characterised by its richness in terpenoid compounds (reviewed by Mumm and Hilker 2006).

To address the question of whether birds respond behaviourally to herbivore-induced cues of conifers, we tested whether wild-caught and temporarily caged Great Tits (*Parus major*) and Blue Tits (*Cyanistes caeruleus*) discriminate between undamaged Scots pine (*Pinus sylvestris*) branches and those that had been systemically damaged by larvae of the pine sawfly (*Diprion pini*). Scots pine is one of the most common coniferous trees worldwide (Carlisle and Brown 1968). Pine sawflies may cause severe damage in pine forests since they can generate high population densities (Géri 1988). Because of active defensive behaviour and distasteful terpenoids, the larvae of the pine sawfly are clearly unpalatable for Great and Blue Tits, unless there is no palatable alternative prey available (Gibb and Betts 1963; Codella and Raffa 1993; Ihalainen et al. 2006; Lindstedt et al. 2011; Barnett et al. 2014).

Assuming an ability to discriminate undamaged and systematically herbivore-induced branches of Scots pine, we expected Great and Blue Tits to go first to or stay longer at the systemically damaged branches. We also investigated whether the branches displayed visual or olfactory cues that the birds could use when exploring the branches.

Methods

Plant and animal species

The bird study was conducted at the Freie Universität Berlin, Germany, in September–October 2012. The HIPV collections were done in spring 2013 and light reflectances were measured in spring/summer 2014. Branches of Scots pine (*P. sylvestris*) were collected from ca. 15-year-old trees growing in the surroundings of Berlin. Scots pines of this age are known to be attacked by sawfly larvae. Branches were taken from those parts of the trees that receive direct sunlight. New branches were cut from the trees every 4–5 weeks (every branch was cut from a different tree during one collection trip). In the laboratory, the cut end of the branches was cleaned according to a method of Moore and Clark (1968). Thereafter, branches were transferred to a climate chamber where they were stored at 10 °C until they were used for the behavioural experiments, light reflectance measurements or HIPV collections. All branches were kept in fresh water throughout the storage and the experiments.

Pine sawflies *(D. pini)* were reared in the laboratory following the methods described by Bombosch and Ramakers (1976) on branches of Scots pine.

The Great and Blue Tits (*P. major* and *C. caeruleus*) were caught with mist nets (mesh: 16 × 16 mm) from gardens and parks close to the experimental setting. Captured birds were tested as soon as possible after capture [time between capture and test was 62.5 (32.5, 122.5) min; median (lower quartile, upper quartile)]. Birds were captured between 7 a.m. and 4 p.m., only if there was no rain, and the number of birds caught daily was 5.3 ± 2.3 (mean ± SD). Captured birds were most probably residents of the area but especially in the later study period some of them could have been autumnal migrants passing through. After the experiment, each bird's sex (male or female) and age (hatched that year or before, i.e. adults or independent juveniles) were determined, wing length and weight were measured, and the bird was ringed. Afterwards, each bird was immediately released close to the place of capture. The time in captivity for each bird was 80.0 (50.0, 141.0) min; median (lower quartile, upper quartile). No bird was used twice for the experiments; birds that were caught a second time and had already been tested were immediately released. Neither the catching method nor the experimental procedure ever caused damage or signs of severe distress to the birds.

Study site for behavioural experiments

All behavioural experiments were conducted in a booth located in a separate experimental room (temperature during experiments: approximately 20 °C). The booth was made of plywood with a smooth surface (width: 100 cm, depth: 100 cm, height: 170 cm). The front wall worked as a door that was opened to prepare the booth with branches prior to the experiment and to recapture the bird after it. A small window (15 × 15 cm) in the door allowed the observation of birds inside the booth. A bird was released into the booth through a small hatch also located in the centre of the door. In the middle of the ceiling was a small round hole (covered with a glass) that was used to record the bird behaviour with a video camera. The booth was lit by a non-flickering True-Light 14-W fluorescent lamp closely resembling the spectrum of natural light including UV wavelengths (importer: Licht + Funktion, Berlin, Germany).

Plant treatment for behavioural experiments

For the choice experiments, two Scots pine branches were prepared for each study day. The branches were of similar size and shape (approximately 70–80 cm long and 30–40 cm wide). Three days before the bird experiment the branches were transferred from the storage climate chamber (10 °C) to a rearing climate chamber (20 °C) for acclimation. Both climate chambers had the same day/night lighting (18/6 h). Both branches had one twig in the lower part covered with a mesh bag (Fig. 1). One of the

Fig. 1 Drawing of a Scots pine (*Pinus sylvestris*) test branch before the choice tests with the birds. In the herbivore-induced branches, pine sawfly larvae (*Diprion pini*) were feeding inside the mesh bag on the lower part of the branch. In the uninfested control branches, the mesh bag was left empty. The twig with the mesh bag was cut off from both herbivore-induced and control branches before the bird tests, and the remaining branch was offered to the birds

two branches was randomly chosen as a test branch subjected to herbivory, and the other one was left undamaged as a control branch.

To obtain a systemically feeding-induced test branch, we placed 30 pine sawfly larvae (about 1 week old) into the mesh bag where they could feed upon the needles for 3 days. The control branch only had the empty mesh bag. After 3 days, in the morning of the experiment, the larvae were gently removed from the test branch, and the twig with the mesh bag was cut off from the test branch. Hence, the test branch had no visible signs of herbivory anymore when used for the experiments, but had been exposed to pine sawfly larval herbivory for 3 days and thus was considered a systemically herbivore-induced test branch. The bagged twig of the control branch was cut off as well before the control branch was used for the behavioural experiment. Thus, except for the herbivore treatment, the test and control branches were subjected to the same conditions. When transferring the test and control branch to the experimental booth, we paid attention to not damaging the needles by handling.

A test branch and a control branch were positioned upright in the booth so that they did not touch each other

(20–50 cm space in between the twig tips) in the experimental booth (side was randomised across study days). In a natural habitat, systemically herbivore-infested trees and non-infested ones might even contact each other. Nevertheless, if the odour of an herbivore-induced branch can help the bird to orient to its prey, we would expect the bird to locate the source from which the odour is emitted. The experimental booth door was widely opened after testing each bird so that it was ventilated with fresh air prior to introducing the next bird.

Behavioural studies

All birds caught during a day were tested with one branch pair consisting of a test and a control branch. The booth was cleaned by wiping with a damp cloth (only water) between the trials if necessary. Only in few instances did the actual branches require gentle cleaning. Damage of needles induces a change of pine odour (Niinemets et al. 2011). The birds did not damage the needles when hopping on the branches and searching between the needles. The position of branches was not changed within 1 day because we wanted to minimise any unwanted damage to the needles that could have resulted from moving them around several times.

Behavioural studies were conducted during 19 days between 5 September and 26 October (i.e. time span 52 days). Each study day we tested 1–9 birds. The branch pair was included as a factor in the statistical tests in order to account for the multiple testing of branches. In total, we tested $n = 68$ Great Tits (41 males and 27 females, 31 adults and 37 juveniles) and $n = 32$ Blue Tits (20 males and 12 females, 17 adults and 15 juveniles) for the two-choice experiments described below.

After being introduced to the booth, birds either flew for a short while or settled on one of the two branches or elsewhere in the booth. Since this very first location often happened immediately after entering and was not preceded by any obvious explorative behaviour, we did not count this first landing as an active choice. Instead, the beginning of an experiment was marked by a conspicuous behaviour: one very short erection and ruffling of the entire body feathers, including the head feathers. This feather ruffle was very easy for the observer to notice. Though the detailed function of this quick feather ruffle has not yet been investigated, it is most probably a sign of tension release or calming (Morris 1956). Linking the feather ruffle with calming is in accordance with our experiments, since after the feather ruffle the birds clearly calmed down (i.e. seemed less nervous and began observing their surroundings) and appeared to start active exploratory behaviour. The quick feather ruffle was shown by almost all birds in our experiments (seven individuals did not ruffle their

feathers, six Great Tits and one Blue Tit; those were excluded from further analyses). A similar behaviour was observed in previous experiments using similar settings and was also considered as the starting point of active food searching (Mäntylä et al. 2004, 2008b). This ruffling behaviour can be reliably determined and usually occurred soon after a bird was introduced to the box [79.0 (44.0, 167.0) s; median (lower quartile, upper quartile)]. Because tit individuals differ in their exploratory behaviour (Dingemanse et al. 2002; Herborn et al. 2010), we decided to use this individual calming down point instead of some pre-determined calming down time. After calming down (as indicated by ruffling) and moving from one branch to another, the birds clearly searched for something hidden between the needles, but in general the birds quite quickly lost their interest in the branches when they could not find any food on them.

The bird's behaviour was recorded with a video camera during the whole stay in the box (calming down period + approximately 15 min of actual test). These recordings were used for later analysis, but also for live observation of the bird's behaviour. If the bird did not show any ruffling behaviour, the test was finished after 15 min. The analysis of all the video recordings was done blind, i.e. the observer did not know which branch was the systemically herbivore-induced test branch and which one was the control. All data were collected by the same person (EM).

From the video recordings we determined the following response patterns: the location of the bird when it was ruffling (either branch or elsewhere), the first choice of the bird (i.e. the first branch it jumped or flew to after ruffling), how many times it visited either of the two branches and how much time it spent on those branches. We calculated these data separately for a period of two and 5 min after the first choice. The first choice has been used as a sign of interest also in other bird studies (Bonadonna and Nevitt 2004; Mardon and Bonadonna 2009).

Collection and chemical analysis of herbivore-induced plant volatiles (HIPVs)

To determine whether different volatile blends of test and control branches would provide cues for the birds to discriminate between them, volatiles were collected from Scots pine branches that had been treated with the same protocol as in the bird experiment. However, the branches were not the same branches the birds were tested with. The HIPVs were collected from untreated control branches and from the undamaged part of the test branches that had only experienced the systemic induction by sawfly larval herbivory. We used $n = 6$ of each systemically herbivore-induced and control branches for the HIPV collection.

The method used for the collection of HIPVs from systemically herbivore-induced and control branches followed the methods used by Mumm et al. (2003) who compared the volatiles released from systemically pine sawfly oviposition-induced and control Scots pine twigs. In spite of the high variability of volatile emissions, this method succeeded in detecting small but consistent differences between the quantitative emission rates of volatiles from oviposition-induced and control Scots pine twigs. A similar method was also successfully applied by Wegener et al. (2001) when comparing the emitted volatiles of oviposition-induced elm (*Ulmus minor*) twigs with control twigs. The comparisons of the blends of volatiles released from the oviposition-induced and the control twigs allowed detecting those volatile compounds that are important for insect orientation towards Scots pine and elm twigs (reviewed by Hilker and Meiners 2010). Hence, although the branches were cut from the tree, they emitted a treatment-specific odour of ecological relevance. Moreover, the odour of insect-treated cut Scots pine twigs was shown to be specific for the Scots pine and insect species (Mumm et al. 2005).

Here, we enclosed a part of a Scots pine branch (i.e. twig) in a bag made of a polyethylene terephthalate (PET) film (Toppits® Bratschlauch, Melitta, Minden, Germany). We bagged the systemically feeding-induced part of the test branch and the respective part of a control branch. The enclosed volume was approximately 1.5 l (30 cm in length, about 15 cm in diameter at the centre). A Teflon tube (5 mm outer diameter) was attached to the PET bag near the base of the twig, and charcoal-purified air was pumped at a flow rate of $6.6 \text{ l} \times \text{h}^{-1}$ into the PET bag. Near the tip of the twig air was sucked out of the bag at a flow rate of $6.0 \text{ l} \times \text{h}^{-1}$ through an adsorption tube (charcoal filter 5 mg, Gränicher and Quartero, Daumazan, France) that was connected via a Teflon tube to a small vacuum pump (Neolab, Heidelberg, Germany). Scots pine volatiles were sampled on the adsorption tube for 2 h in the afternoon at the 20 °C climate chamber (day/night 18/6 h). Sampled volatiles were eluted from the adsorption tube with 25 µl dichloromethane containing $125 \text{ ng} \times \text{µl}^{-1}$ methyl octanoate (Sigma-Aldrich, Deisenhofen, Germany) as an internal standard.

Samples were analysed by coupled gas chromatography-mass spectrometry (GC-MS) (Fisons 8060 GC with Fisons MD800 quadrupole mass spectrometer). The injection volume was 1 µl (splitless). The volatiles were separated on a 30 m × 0.32 mm i.d. DB-5MS fused silica column, film thickness 0.25 µm (J&W Scientific, Folsom, CA) with helium as carrier gas (head pressure 10 kPa). The temperature was initially held at 40 °C for 4 min and then increased at $3 \text{ °C} \times \text{min}^{-1}$ to 280 °C. An electron impact ionisation (EI) source was used with 70 eV ionisation

voltage. Linear retention indices were estimated by co-injection of a hydrocarbon mixture (C7–C20) and calculated according to van den Dool and Kratz (1963). The identification of compounds was based on comparison of mass spectra with those of authentic reference compounds or with NIST and Wiley library spectra. Retention indices were compared to data from NIST Chemistry WebBook (Stein 2011) and the terpenoid library of MassFinder 4 (König et al. 2006). Relative quantities of compounds were determined by relating the peak area of each compound to the peak area of the internal standard (=100%; 125 ng × μl^{-1} methyl octanoate).

Analysis of Scots pine needle light reflectance by spectrophotometer measurements and discrimination threshold model for Blue Tit vision

To determine whether birds can detect potential colour differences between the systemically herbivore-induced and undamaged control Scots pine branches, we determined the light reflectance of test and control Scots pine branches and ran a threshold discrimination model (see below).

Light reflectance of systemically herbivore-induced and undamaged control Scots pine branches was measured by using a spectrophotometer (Agilent Cary 60 UV–Vis with Remote Diffuse Reflectance Accessory). For these measurements, we prepared Scots pine branches in a similar way as for the HIPV collection. For each scan (300–700 nm) the needles of a branch were bundled (without removing them from the twig) and pushed together to obtain as planar a surface as possible. This scan procedure was repeated at least 20 times for each branch. In total, we measured the reflectance of each $n = 7$ systemically herbivore-induced test branches and control branches.

We used discrimination threshold modelling (Blue Tit vision) to compare the colour and luminance of the systemically herbivore-induced and undamaged control pine branches. The discrimination threshold model used assumes that noise in the receptors limits the visual discrimination ability (Vorobyev and Osorio 1998; Vorobyev et al. 1998) of the birds. The model uses information about the visual system such as the sensitivity and relative abundance of different photoreceptor types and estimates of noise that arise in the photoreceptors. The model is for the Blue Tit, but Great Tits are expected to have a similar visual perception since the species are close relatives that live in the same environment and forage for similar prey. Average spectra were taken for each control and systemically herbivore-induced Scots pine branch, followed by modelling of a Blue Tit's photon catch values for the single

and double cones (Hart et al. 2000) with a standard D65 irradiance spectrum. Colour vision in birds is enabled by four single-cone types [i.e. long wave (LW), medium wave (MW), short wave (SW) and ultraviolet sensitive (UV); Cuthill 2006], whereas luminance discrimination apparently is enabled by the double cones (D; Osorio and Vorobyev 2005). For the colour discrimination model, we therefore used the four single cones, whereas the luminance discrimination model was based on the double cones.

For the discrimination model, we used a Weber fraction of 0.05 for the most abundant retinal cone type and the relative proportions of cone types in the Blue Tit retina (LW = 1.00, MW = 0.99, SW = 0.71 and UV = 0.37). For the results of the discrimination model, values for both colour and luminance (just noticeable differences, or 'JNDs') of less than 1 mean that birds are not able to tell control and experimental branch apart, values between 1 and 3 are hard to distinguish unless under optimal conditions, and values of more than 3 are easy to tell apart under most conditions.

Statistical analyses

We used chi-square analyses to determine whether the birds' behavioural responses to test and control Scots pine branches significantly differed with respect to their first choice, the ruffle place, first landing and the visited place at a fixed time point (see below for more details of these).

To further analyse the data on bird behaviour, we calculated generalised linear mixed models (GLMM) using a residual pseudo-likelihood estimation method to test whether the first choice of the bird (herbivore or control branch) was affected by any of the following factors: sex (male or female), age (adult or juvenile), species (Great Tit or Blue Tit), position of the herbivore branch (left or right), place where the bird calmed down (i.e. ruffle place: herbivore branch, control branch or elsewhere) or running number of date [day 1 is the first test day (5 September) and day 52 the last one (26 October)]. Binomial distributions with logit link functions were used in the GLIMMIX procedure of the SAS statistical software, version 9.4. The lack of the degrees of freedom (df) prevented us from testing possible interactions. To ensure complete independence across the subjects, the branch pair was used as a subject and a random effect in the RANDOM statement. To compute the denominator df, we divided the residual df into between- and within-subject portions (option BETWITHIN).

For the analysis of the chemical data, we used the Wilcoxon signed rank test (the UNIVARIATE procedure of SAS) to test for differences between treatments in the quantitative emission of each HIPV (in total 29 compounds). We used this non-parametric test because of the non-normal distributions of the quantities of many of the

detected HIPVs. A correction for multiple testing was applied as described by Benjamini and Hochberg (1995), and *p* values were adjusted accordingly with the equation given by Dudoit et al. (2003) to control the false discovery rate at an alpha level of 5%.

The spectrophotometer data were analysed with linear mixed models (the MIXED procedure of SAS). We analysed the differences in light reflectance (total reflectance sum, i.e. brightness) between herbivore-induced and control branches separately for 300–700 nm (avian vision range), 300–400 nm (UV light range) and 400–700 nm (human vision range). The identity of the branch was used as a clustering factor (REPEATED statement) to control the fact that the 20 measurements from the same branch were not independent observations. We used a general Satterthwaite approximation (option SATTERTHWAITE) as the method for computing the denominator *df*.

Results

Behavioural studies

When having the choice between a systematically herbivore-induced test branch and a control branch, Great Tits and Blue Tits ($N = 93$ birds tested in total) significantly more often visited the test branch first after calming down (62 vs. 31; $\chi^2 = 10.3$, $p = 0.001$; Fig. 2a, b). There was no effect of bird species, sex or age on this choice (Table 1), nor did the side where the herbivore branch was located in the booth (right/left) or the running number of the date the bird was tested affect this result (Table 1).

We further explored how the preference for the herbivore-induced branch was related to (1) the place where a bird calmed down, (2) the first branch a bird landed on after release in the booth and (3) a pre-determined time (1 min) for relaxing after release into the booth.

1. After the birds had been released to the experimental booth, they did not show a preference for the herbivore-induced or the control branch as the calming down place (herbivore 37 vs. control 27; $\chi^2 = 1.26$, $p = 0.26$; Table 2). However, the place where the bird was while calming down (i.e. quick feather ruffle) had an effect on the following first choice of the bird (Table 1). If the bird was sitting on the systemically herbivore-induced test branch when ruffling, its first choice often was also the test branch. But if the bird was sitting on the control branch or elsewhere in the booth when ruffling, there was no difference in its first choice after feather ruffling (Table 2). After the first choice the bird remained on that branch for 58.2 ± 9.9 s (mean $\pm$ SE) before moving to another branch or elsewhere. There was no difference in the duration of the first visit between test and control branches ($t = 1.06$, $df = 88$, $p = 0.29$). Also the total number of visits (Fig. 3a) and the total time spent (Fig. 3b) on test and control branches did not differ.

2. When the birds were landing on either branch for the first time after release to the booth, this landing happened with the same probability on herbivore-induced and control branches (herbivore 53 vs. control 46, $\chi^2 = 0.49$, $p = 0.48$, one bird did not sit on any branch during the 15 min). However, in several cases the first landing was actually the same visit as the first
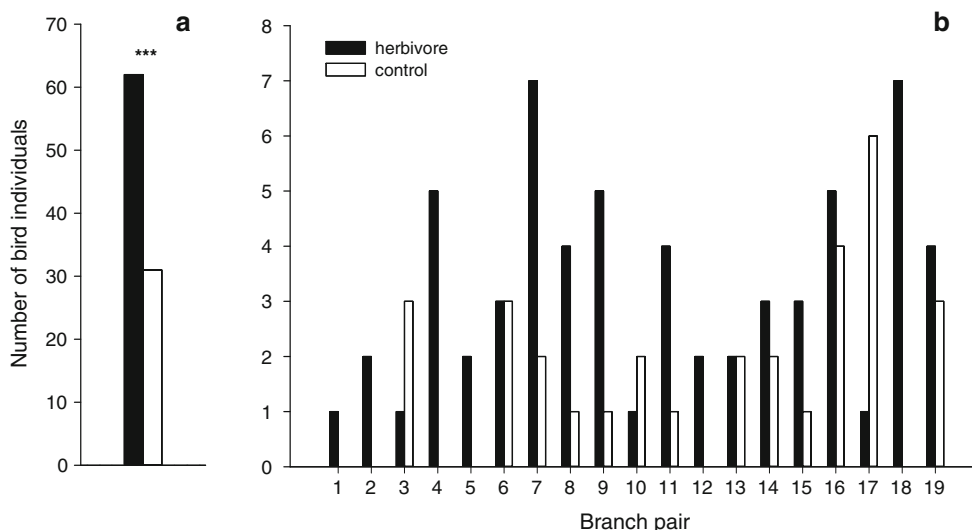

Fig. 2 a The total number of individuals per branch pair that chose a systemically herbivore-induced Scots pine branch (*black bar*, n = 62) or a control branch (*white bar*, n = 31) (***p = 0.001). **b** The same data separately for every branch pair (a new branch pair was used for every study day). The *numbers of the branch pairs* show the order in which they were used in the experiment

Table 1 Result of the generalised linear mixed model (GLMM) on fixed factors affecting the first choice of the birds

Parameter[a]	F	df	p
Species	0.02	1, 12	0.90
Sex	0.93	1, 14	0.35
Age	1.80	1, 13	0.20
Side	2.10	1, 16	0.17
Date	1.93	1, 16	0.18
Ruffle place	6.05	2, 27	**0.007**

Significant *p*-value is shown in bold

[a] [Species = Great Tit or Blue Tit; sex = male or female; age = adult or juvenile; side = position of the herbivore branch in the booth (right or left); date = running number of date 1–52; ruffle place = place where the bird calmed down (herbivore branch, control branch or elsewhere)]. The identity of the branch pair was used as a random factor

choice after calming down (herbivore 31 and control 17). This happened either when the bird calmed down elsewhere and visited a branch the first time after that or when the bird first landed on a branch, calmed down on it and hopped on other part of the same branch without leaving the branch between those actions. The birds landed on 1.1 ± 0.2 (mean ± SE; minimum 0, maximum 13) branches before the first choice visit after calming down. Many of the birds were flying around in the booth when released there; it looked as if they were accidentally landing on some branch. Therefore, we decided to record which branch was visited first by a bird after calming down in the booth.

3. To further justify the use of feather ruffling as a sign of calming down after which choice behaviour would be possible, we analysed the data alternatively by choosing a fixed time (1 min after release to the booth) and observed which branch they first jumped on or flew to after that time. There was again no statistical difference (herbivore 55 vs. control 44, $\chi^2 = 1.22$, $p = 0.27$), which is well explainable when considering the time until calming down. Although the median calming down duration was 79 s after release to the booth, there was huge variation in it. A total of 35 birds

calmed down (i.e. ruffled their feathers) in less than 60 s and most of them at that point were already jumping from branch to branch, so the time point of 60 s missed their calming down point and first choice. On the other hand, for 13 birds it took more than 5 min to calm down. These birds were usually sitting in one place before calming down.

Hence, birds showed only a clear preference for the herbivore-induced branch after having calmed down. This preference was not detectable when analysing which type of branch was preferred for the first landing (and for calming down) after release into the booth, nor was a preference for herbivore-induced branches detectable when analysing the data after a fixed time of release into the booth because the time that the birds needed to calm down (and show preference behaviour) varied greatly.

As a first choice after calming down, the test branch was preferred over the control branch on 14 study days and vice versa on 3 study days ($\chi^2 = 5.88$, $p = 0.015$; Fig. 2b). On 2 study days there were equal numbers of first choices of both branches. Since we tested 1–9 birds per day without changing the branches in the booth, the first choice by the first bird tested per day might have affected the first choices of birds tested later on the same day. However, when considering the behaviour of only the first birds tested on a study day (i.e. birds whose behaviour could not have been affected by prior visits of the branch by other birds), the majority of them (12 individuals) made their first choice for the systemically herbivore-induced branch and four for the control branch ($\chi^2 = 3.06$, $p = 0.08$). On 3 days the first bird did not calm down.

HIPV emissions

HIPV collections were conducted to identify odour differences between test and control branches that might potentially account for attraction of birds to the branches. We detected 29 volatile compounds in the headspace of test and control branches, and 21 of these compounds were emitted in significantly greater quantities from the systemically herbivore-induced branches than from the control branches (Fig. 4).

Table 2 The place where the birds calmed down (=ruffle place, on either branch or elsewhere, i.e. on the floor or on the pots where the branches were placed) and first choice of the birds after calming down (herbivore branch or control branch)

Ruffle place	First choice	No. of individuals	χ^2	df	p*
Herbivore branch	Herbivore branch	33	22.73	1	**<0.0001**
	Control branch	4			
Control branch	Herbivore branch	14	0.04	1	1.00
	Control branch	13			
Elsewhere	Herbivore branch	15	0.03	1	1.00
	Control branch	14			

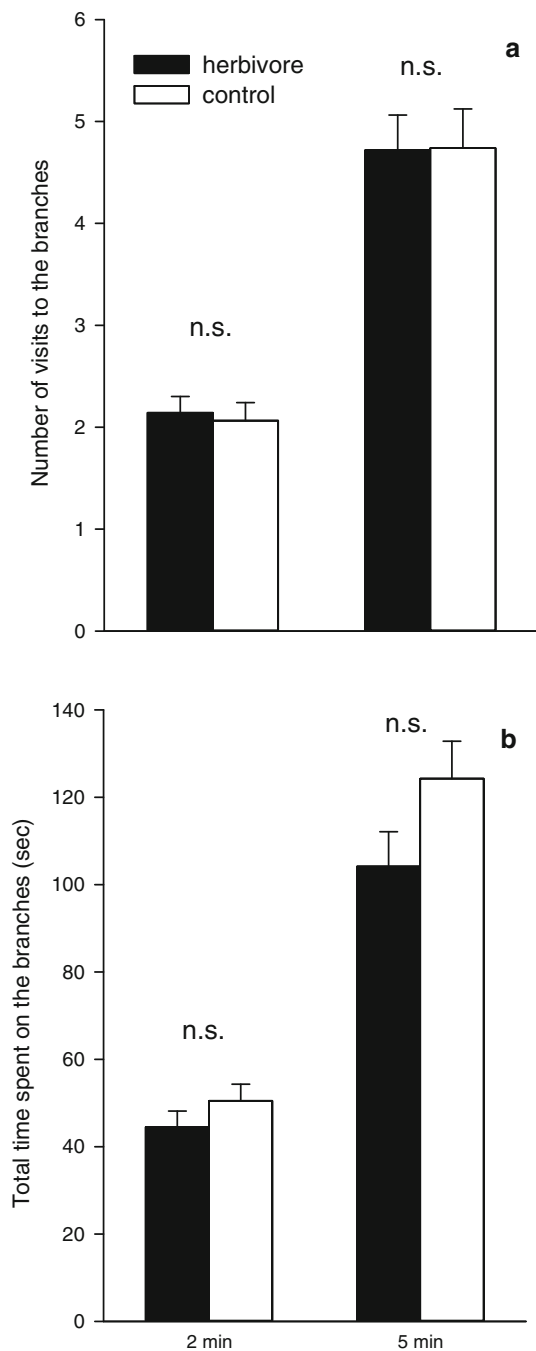Significant *p*-value is shown in bold

* Kruskal-Wallis tests

Fig. 3 **a** The number of visits made by the birds to the systemically herbivore-induced and the untreated control branches (2 min: $t = 0.00$, $df = 90$, $p = 1.00$; 5 min: $t = -0.08$, $df = 90$, $p = 0.94$) and **b** the total time the birds spent (2 min: $t = -0.89$, $df = 90$, $p = 0.38$; 5 min: $t = -1.33$, $df = 90$, $p = 0.19$) on those two branches during the 2 and 5 min after the first choice (paired t test; mean $\pm$ SE)

Light reflectance and discrimination threshold model

We measured light reflectance of needles of test and control branches to find out if birds could recognise these through

visual cues. Throughout the avian vision range of wavelengths (300−700 nm), the needles of the undamaged control branches reflected somewhat more light than those of the systemically herbivore-induced test branches (Fig. 5), but this difference was not statistically significant (300–700 nm: $F_{df} = 1.97_{1,12}$, $p = 0.186$; 300–400 nm: $F_{df} = 2.17_{1,11.9}$, $p = 0.167$; 400–700 nm: $F_{df} = 1.78_{1,12}$, $p = 0.207$). Similarly, the threshold model suggests that the difference in colour (JND = 0.6) and luminance (JND = 0.83) is not visible for birds (Fig. 6).

Discussion

Our study shows that both Great Tits and Blue Tits were attracted to coniferous Scots pine branches systemically induced by feeding pine sawfly larvae, although the birds could neither see nor smell the larvae, their faeces or damaged needles. This attraction was observed when birds first had the chance to calm down in an experimental booth before their preference behaviour and first choice of a branch was recorded. Our analyses revealed that systemically feeding-induced test Scots pine branches and undamaged control branches differed significantly in their volatile emission, while differences in their spectral reflectance did not look different for birds. Thus, birds may have used at least olfaction for discrimination between test and control branches.

When analysing the behaviour of the birds during the first 2 and 5 min of the experiment, the number of visits to the branches or time spent on the branches did not differ. Only the first choice was significantly more often made for the herbivore-induced branch than for the control branch. These results indicate that the birds were attracted to the systemically herbivore-induced branch, but quickly lost their interest in a particular branch when they did not find any food on those. Usually quite soon after the first choice the birds were just hopping from one branch to another and not searching for anything between the needles anymore.

Because the birds' interest in the branches had in most cases a very short duration, it was important to discern this time of interest for each individual. We observed in this study—similar to observations in earlier studies (Mäntylä et al. 2004, 2008b)—that the birds became relaxed and more active after a quick feather ruffle soon after entering the study booth. Therefore, we chose this as the time of calming down and starting to monitoring the birds' preference behaviour. It is known that exploratory behaviour of Great Tits and Blue Tits is individually consistent; some individuals are bolder in exploring new environments than others (Verbeek et al. 1994; Dingemanse et al. 2002; Herborn et al. 2010). After release into an experimental booth, the bird's first landing place was obviously chosen
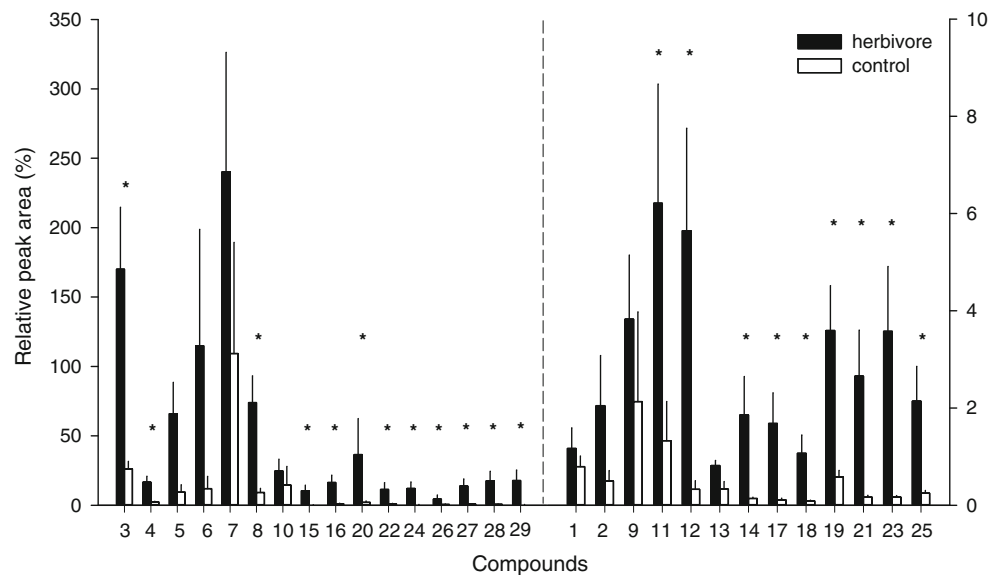
Fig. 4 HIPV emissions (mean ± SE) from *P. sylvestris* branches systemically induced by feeding of pine sawfly larvae *D. pini* (*n* = 6) and untreated control branches (*n* = 6). The HIPV emissions were compared to an internal standard (methyl octanoate; 100% = 3.125 μg). Notice the two different *y*-axes. (Wilcoxon signed rank test with Benjamini-Hochberg correction: *$p < 0.05$). Compounds: *1* hexanal, *2* (Z)-3-hexen-1-ol, *3* α-pinene, *4* camphene, *5* sabinene + β-pinene, *6* myrcene, *7* δ-3-carene, *8* β-phellandrene, *9* γ-terpinene, *10* terpinolene, *11* isobornyl acetate, *12* bicycloelemene, *13* δ-elemene, *14* α-copaene, *15* β-elemene, *16* (E)-β-caryophyllene, *17* isogermacrene D, *18* aromadendrene, *19* α-humulene, *20* (E)-β-farnesene, *21* α-amorphene, *22* germacrene D, *23* α-selinene, *24* bicyclogermacrene, *25* α-muurolene, *26* α-farnesene, *27* γ-cadinene, *28* δ-cadinene, *29* germacrene-D-4-ol
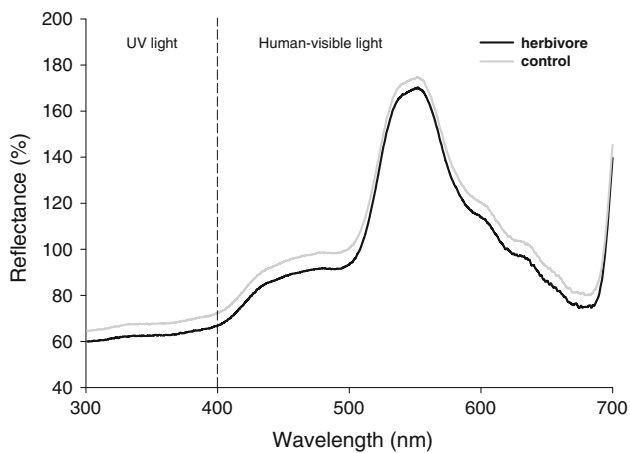

Fig. 5 Light reflectance curves of Scots pine needles from systemically herbivore-induced (*black*) and control branches (*grey*) in the avian vision range (300–700 nm). The *lines* show mean values of both treatments. The light reflectance could rise above 100% because the spectrophotometer was calibrated with the white standard

Fig. 6 The mean cone catch values of Blue Tit vision [(UV + SW + MW + LW + D)/5] of herbivore-induced (mean = 0.0109, SD = 0.0009, *n* = 7) and control (mean = 0.0115, SD = 0.0008, *n* = 7) branches. The *horizontal lines* show the mean of the herbivore-induced and the control treatments

just by chance. Thus, recording only the branch where birds landed first does not provide information on the birds' choices when foraging for a resource. Recording of bird behaviour after a fixed time period following release into the experimental booth also does not provide valuable data on the birds' foraging behaviour because each bird needs a different time to calm down in the booth, and calming down after release is considered a prerequisite to starting foraging and making choices.
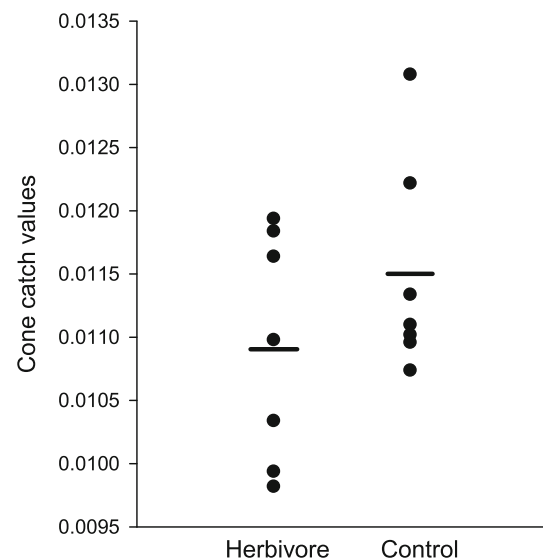
While the first-choice preference for the herbivore-induced branch was not affected by the bird's species, sex, age or experimental factors such as the branch location side or running number of the date, the site where the birds calmed down affected their first choice thereafter. However, the birds did not prefer the herbivore-induced branch as a place

for calming down. The preference of birds to further search for the herbivore-induced branch (i.e. first choice) after having calmed down on this branch shows that the herbivore-induced cues are most likely clear and easy to detect.

We also asked whether there is any evidence that the first-choice preference for the herbivore-induced branch might have been affected by the previous visits of other bird individuals tested on the same day on the same branches. However, the majority of birds that were tested first on a study day chose first the herbivore-induced branch (12), while only a few (4) chose the control branch; the lack of a statistically significant difference in the frequency of these choices is probably due to the relatively low numbers that are compared. Hence, our data indicate that the first-choice preference for the herbivore-induced branches is not affected by prior visits by other individuals.

Earlier studies on the attraction of birds to herbivore-damaged trees have shown that birds could possibly smell and use the following volatile compounds as cues: (E)-DMNT, β-ocimene and linalool (Mäntylä et al. 2008a), α-farnesene (Amo et al. 2013) and α-pinene (Mäntylä et al. 2014). Of these compounds in our study we found α-farnesene and α-pinene to be compounds emitted in significantly higher quantities by the systemically herbivore-induced than by the control Scots pine branches. Thus, it is possible that the birds used α-farnesene and/or α-pinene—probably in concert with other volatiles—to locate the herbivore-induced branches. Undamaged Scots pines also emit volatiles (e.g. α-pinene, camphene, β-pinene, myrcene and terpinolene), but feeding by pine sawfly larvae increases these amounts significantly (Ghimire et al. 2013). In our study we found a similar difference in the emission of those and several other compounds.

In our study we used branches cut from Scots pine trees, and it is possible that the emitted volatiles or their ratios could be different in whole, growing trees. For invertebrates, the attraction to an odour may be determined by the ratio of quantities of certain key compounds within a complex blend of volatiles (reviewed by Beyaert and Hilker 2014). For birds, it is known that pelagic birds are attracted by a single key component, i.e. dimethyl sulphide emitted from areas rich in phytoplankton as food (Nevitt et al. 1995; Savoca and Nevitt 2014). However, it is possible that in other olfactory tasks a correct quantitative blend of several volatile compounds is needed for recognition. Future studies need to elucidate whether this is also the case for the attractiveness of systemically herbivore-induced Scots pine trees and whether the ratio of α-farnesene and/or α-pinene quantities to other compounds is important in a natural context with trees. Our current study indicates that birds are able to discriminate between the odour of systemically herbivore-induced Scots pine branches and insect-free Scots pine branches. Whether birds indeed use the odour of systemically herbivore-induced

Scots pine trees when searching for prey and whether orientation towards this odour helps locate the prey need to be investigated by field experiments.

The light reflectance measurements were highly variable both within and between branches. Thus, subtle differences between test and control branches might have been lost because of the statistical power. Hence, we cannot exclude that the birds in the current study used their entire range of vision (300–700 nm) to identify herbivore-damaged trees even in the absence of visible prey items or damaged leaves or needles, as the light reflectance tended to be higher in control branches than in herbivore-induced branches throughout that range.

So far, we do not know yet how the learning ability of tits and the (dis)tastefulness of sawfly larvae affect their attraction to herbivore-induced Scots pine. The pine sawfly larvae (and other Diprionidae species) gather and store terpenoid resin of their host plants in special foregut pouches. The larvae regurgitate this sticky and smelly resin liquid when attacked, and sometimes the predators are repelled by it (Eisner et al. 1974; Codella and Raffa 1996). Great Tits are known to eat in addition to pine sawfly larvae also other distasteful pest insects of pines, such as European pine sawflies (*Neodiprion sertifer*) and pine processionary moths (*Thaumetopoea pityocampa*) (Halperin 1990; Sillén-Tullenberg 1990).

In conclusion, our results strengthen the evidence that passerine birds are attracted to herbivore-damaged trees even when they do not see the physical damage caused by the herbivores. Our analyses suggest that olfaction (and possibly also vision) could be used by the birds to recognise herbivore-infested Scots pines. Future studies need to further elucidate which of the numerous Scots pine volatiles released by herbivore-infested trees growing in the field actually attract the birds and whether visual cues due to light reflectance changes upon herbivory also play a role. Another future study would be to offer a combination of (1) the visual cue of an undamaged branch and (2) the olfactory cue provided by a synthetic blend of the HIPVs detected in the headspace of systemically induced Scots pine to the birds. It would be interesting to elucidate whether this combination is preferred to an undamaged branch without additional synthetic scent. Since exposure of plants to HIPVs is known to induce odour changes in the scented plant (Heil and Karban 2010), the combination of visual and olfactory cues needs to be designed so that the HIPVs cannot induce any plant stress odours.

References

Amo L, Galvan I, Tomás G, Sanz JJ (2008) Predator odour recognition and avoidance in a songbird. Funct Ecol 22:289–293

Amo L, Visser ME, van Oers K (2011) Smelling out predators is innate in birds. Ardea 99:177–184

Amo L, Jansen JJ, van Dam NM, Dicke M, Visser ME (2013) Birds exploit herbivore-induced plant volatiles to locate herbivorous prey. Ecol Lett 16:1348–1355

Amo L, Dicke M, Visser ME (2016) Are naïve birds attracted to herbivore-induced plant defences? Behaviour 153:353–366

Bansal S, Hallsby G, Löfvenius MO, Nilsson M-C (2013) Synergistic, additive and antagonistic impacts of drought and herbivory on *Pinus sylvestris*: leaf, tissue and whole-plant responses and recovery. Tree Physiol 33:451–463

Barnett CA, Bateson M, Rowe C (2014) Better the devil you know: avian predators find variation in prey toxicity aversive. Biol Lett 10:20140533

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Statist Soc B 57:289–300

Beyaert I, Hilker M (2014) Plant odour plumes as mediators of plant-insect interactions. Biol Rev 89:68–81

Blande JD, Turunen K, Holopainen JK (2009) Pine weevil feeding on Norway spruce bark has a stronger impact on needle VOC emissions than enhanced ultraviolet-B radiation. Environ Pollut 157:174–180

Bombosch S, Ramakers PMJ (1976) Zur Dauerzucht von *Gilpinia hercyniae* (Htg). Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 83:40–44

Bonadonna F, Nevitt GA (2004) Partner-specific odour recognition in an Antarctic seabird. Science 306:835

Carlisle A, Brown AHF (1968) *Pinus sylvestris*. J Ecol 56:269–307

Codella SG, Raffa KF (1993) Defense strategies of folivorous sawflies. In: Wagner MR, Raffa KF (eds) Sawfly life history adaptations to woody plants. Academic Press, San Diego, pp 261–294

Codella SG, Raffa KF (1996) Individual and social components of wood ant response to conifer sawfly defence (Hymenoptera: Formicidae, Diprionidae). Anim Behav 52:801–811

Copolovici L, Kannaste A, Remmel T, Vislap V, Niinemets Ü (2011) Volatile emissions from *Alnus glutionosa* induced by herbivory are quantitatively related to the extent of damage. J Chem Ecol 37:18–28

Cuthill IC (2006) Color perception. In: Hill GE, McGraw KJ (eds) Bird coloration, mechanisms and measurements. Harvard University Press, Cambridge, pp 3–40

D'Alessandro M, Turlings TCJ (2006) Advances and challenges in the identification of volatiles that mediate interactions among plants and arthropods. Analyst 131:24–32

Dicke M (2009) Behavioural and community ecology of plants that cry for help. Plant Cell Environ 32:654–665

Dingemanse NJ, Both C, Drent PJ, van Oers K, van Noordwijk A (2002) Repeatability and heritability of exploratory behaviour in great tits from the wild. Anim Behav 64:929–938

Dudoit S, Shaffer JP, Boldrick JC (2003) Multiple hypothesis testing in microarray experiments. Statist Sci 18:71–103

Eisner T, Johnessee JS, Carrel J, Hendry LB, Meinwald J (1974) Defensive use by an insect of a plant resin. Science 184:996–999

Eyles A, Smith D, Pinkard EA, Smith I, Corkrey R, Elms S, Beadle C, Mohammed C (2011) Photosynthetic responses of field-grown *Pinus radiata* trees to artificial and aphid-induced defoliation. Tree Physiol 31:592–603

Gagliardo A (2013) Forty years of olfactory navigation in birds. J Exp Biol 216:2165–2171

Géri C (1988) The pine sawfly in central France. In: Berryman AA (ed) dynamics of forest insect populations. Patterns, causes, implications. Plenum Press, New York, pp 377–405

Ghimire RP, Markkanen M, Kivimäenpää M, Lyytikäinen-Saarenmaa P, Holopainen JK (2013) Needle removal by pine sawfly larvae increases branch-level VOC emissions and reduces below-ground emissions of Scots pine. Environ Sci Technol 47:4325–4332

Gibb JA, Betts MM (1963) Food and food supply of nestling tits (Paridae) in Breckland pine. J Anim Ecol 32:489–533

Gwinner H, Berger S (2008) Starling males select green nest material by olfaction using experience-independent and experience-dependent cues. Anim Behav 75:971–976

Halperin J (1990) Natural enemies of *Thaumetopoea* spp. (Lep., Thaumetopoeidae) in Israel. J Appl Entomol 109:425–435

Hart NS, Partridge JC, Cuthill IC (2000) Retinal asymmetry in birds. Curr Biol 10:115–117

Heil M, Karban R (2010) Explaining evolution of plant communication by airborne signals. Trends Ecol Evol 25:137–144

Herborn KA, Macleod R, Miles WTS, Schofield ANB, Alexander L, Arnold KE (2010) Personality in captivity reflects personality in the wild. Anim Behav 79:835–843

Hilker M, Meiners T (2010) How do plants "notice" attack by herbivorous arthropods? Biol Rev 85:267–280

Holland RA, Thorup K, Gagliardo A, Bisson IA, Knecht E, Mizrahi D, Wikelski M (2009) Testing the role of sensory systems in the migratory heading of a songbird. J Exp Biol 212:4065–4071

Holopainen JK (2004) Multiple functions of inducible plant volatiles. Trends Plant Sci 9:529–533

Holopainen JK (2012) Can forest trees compensate for stress-generated growth losses by induced production of volatile compounds? Tree Physiol 31:1356–1377

Howe GA, Jander G (2008) Plant immunity to insect herbivores. Annu Rev Plant Biol 59:41–66

Ihalainen E, Lindström L, Mappes J (2006) Investigating Müllerian mimicry: predator learning and variation in prey defences. J Evol Biol 20:780–791

Jones MP, Pierce KE, Ward D (2007) Avian vision: a review of form and function with special consideration to birds of prey. J Exot Pet Med 16:69–87

Karban R, Baldwin IT (1997) Induced Responses to Herbivory. University of Chicago Press, Chicago

König WA, Joulain D, Hochmuth DH (2006) GC/MS library: Terpenoids and Related Constituents of Essential Oils. http://massfinder.com/wiki/Terpenoids_Library. Accessed 18 June 2014

Koski T-M, Laaksonen T, Mäntylä E, Ruuskanen S, Li T, Girón-Calva PS, Huttunen L, Blande JD, Holopainen JK, Klemola T (2015) Do insectivorous birds use volatile organic compounds from plants as olfactory foraging cues? Three experimental tests. Ethology 121:1131–1144

Krause ET, Krüger O, Kohlmeier P, Caspers BA (2012) Olfactory kin recognition in a songbird. Biol Lett 8:327–329

Krause ET, Brummet C, Kohlwey S, Baier MC, Müller C, Bonadonna F, Caspers BA (2014) Differences in olfactory species recognition in the females of two Australian songbird species. Behav Ecol Sociobiol 68:1819–1827

Lind O, Mitkus M, Olsson P, Kelber A (2014) Ultraviolet vision in birds: the importance of transparent eye media. Proc R Soc B Biol Sci 281:20132209

Lindstedt C, Huttunen H, Kakko M, Mappes J (2011) Disentangling the evolution of weak warning signals: high detection risk and low production costs of chemical defences in gregarious pine sawfly larvae. Evol Ecol 2:1029–1046

Mäntylä E, Klemola T, Haukioja E (2004) Attraction of willow warblers to sawfly-damaged mountain birches: novel function of inducible plant defenses? Ecol Lett 7:915–918

Mäntylä E, Alessio GA, Blande JD, Heijari J, Holopainen JK, Laaksonen T, Piirtola P, Klemola T (2008a) From plants to birds: higher avian predation rates in trees responding to insect herbivory. PLoS One 3:e2832

Mäntylä E, Klemola T, Sirkiä P, Laaksonen T (2008b) Low light reflectance may explain the attraction of birds to defoliated trees. Behav Ecol 19:325–330

Mäntylä E, Klemola T, Laaksonen T (2011) Birds help plants: a meta-analysis of top-down trophic cascades caused by avian predators. Oecologia 165:143–151

Mäntylä E, Blande JD, Klemola T (2014) Do birches treated with methyl jasmonate attract insectivorous birds in the nature? Arthropod-Plant Interact 8:143–153

Mardon J, Bonadonna F (2009) Atypical homing or self-odour avoidance? Blue petrels (Halobaena caerulea) are attracted to their mate's odour but avoid their own. Behav Ecol Sociobiol 63:537–542

Mennerat A, Bonadonna F, Perret P, Lambrechts MM (2005) Olfactory conditioning experiments in a food-searching passerine bird in semi-natural conditions. Behav Process 70:264–270

Mooney KA, Gruner DS, Barber NA, Van Bael SA, Philpott SM, Greenberg R (2010) Interactions among predators and the cascading effects of vertebrate insectivores on arthropod communities and plants. Proc Natl Acad Sci USA 107:7335–7340

Moore GE, Clark EW (1968) Suppressing microorganisms and maintaining turgidity in coniferous foliage used to rear insects in laboratory. J Econ Entomol 61:1030–1031

Morris D (1956) The feather postures of birds and the problem of the origin of social signals. Behaviour 9:75–111

Mumm R, Hilker M (2006) Direct and indirect chemical defence of pine against folivorous insects. Trends Plant Sci 11:351–358

Mumm R, Schrank K, Wegener R, Schulz S, Hilker M (2003) Chemical analysis of volatiles emitted by Pinus sylvestris after induction by insect oviposition. J Chem Ecol 29:1235–1252

Mumm R, Tiemann T, Varama M, Hilker M (2005) Choosy egg parasitoids: specificity of oviposition-induced pine volatiles exploited by an egg parasitoid of pine sawfly. Entomol Exp Appl 115:217–225

Nabity PD, Zavala JA, DeLucia EH (2009) Indirect suppression of photosynthesis on individual leaves by arthropod herbivory. Ann Bot 103:655–663

Nevitt GA, Veit RR, Kareiva P (1995) Dimethyl sulphide as a foraging cue for Antarctic procellariiform seabirds. Nature 376:680–682

Niinemets Ü, Kuhn U, Harley PC, Staudt M, Arneth A, Cescatti A, Ciccioli P, Copolovici L, Geron C, Guenther A, Kesselmeier J, Lerdau MT, Monson RK, Peñuelas J (2011) Estimations of isoprenoid emission capacity from enclosure studies: measurements, data processing, quality and standardized measurement protocols. Biogeosciences 8:2209–2246

Nykänen H, Koricheva J (2004) Damaged-induced changes in woody plants and their effects on insect herbivore performance: a meta-analysis. Oikos 104:247–268

Orians C (2005) Herbivores, vascular pathways, and systemic induction: facts and artifacts. J Chem Ecol 31:2231–2242

Osorio D, Vorobyev M (2005) Photoreceptor sectral sensitivities in terrestrial animals: adaptations for luminance and colour vision. Proc R Soc B 272:1745–1752

Peñuelas J, Munné-Bosch S, Llusià J, Filella I (2004) Leaf reflectance and photo- and antioxidant protection in field-grown summer-stressed Phillyrea angustifolia. Optical signals of oxidative stress? New Phytol 162:115–124

Petit C, Hossaert-McKey M, Perret P, Blondel J, Lamberchts MM (2002) Blue tits use selected plants and olfaction to maintain an aromatic environment for nestlings. Ecol Lett 5:585–589

Pinkard EA, Battagla M, Roxburgh S, O'Grady AP (2011) Estimating forest net primary production under changing climate: adding pests into the equation. Tree Physiol 31:686–699

Roper TJ (1999) Olfaction in birds. Adv Stud Behav 28:247–332

Savoca MS, Nevitt GA (2014) Evidence that dimethyl sulfide facilitates a tritrophic mutualism between marine primary producers and top predators. Proc Natl Acad Sci USA 111:4157–4161

Sillén-Tullenberg B (1990) Do predators avoid groups of aposematic prey? An experimental test. Anim Behav 40:856–860

Steiger SS, Fidler AE, Valcu M, Kempenaers B (2008) Avian olfactory receptor gene repertoires: evidence for a well-developed sense of smell in birds? Proc R Soc B Biol Sci 275:2309–2317

Stein SE (2011) Retention indices. In: Linstrom PJ, Mallard WG (eds) NIST Chemistry WebBook, NIST Standard Reference Database Number 69, National Institute of Standards and Technology. Gaithersburg, Md, USA. http://webbook.nist.gov/

Van Bael SA, Philpott SM, Greenberg R, Bichier P, Barber NA, Mooney KA, Gruner DS (2008) Birds as predators in tropical agroforestry systems. Ecology 89:928–934

van den Dool H, Kratz PD (1963) A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr 11:463–471

Vanderklein DW, Reich PB (2000) European larch and eastern white pine respond similarly during three years of partial defoliation. Tree Physiol 20:283–287

Verbeek MEM, Drent PJ, Wiepkema PR (1994) Consistent individual differences in early exploratory behaviour of male great tits. Anim Behav 48:1113–1121

Vorobyev M, Osorio D (1998) Receptor noise as a determinant of colour thresholds. Proc R Soc Lond B 265:351–358

Vorobyev M, Osorio D, Bennett ATD, Marshall NJ, Cuthill IC (1998) Tetrachromacy, oil droplets and bird plumage colors. J Comp Physiol A 183:621–633

Walling LL (2000) The myriad plant responses to herbivores. J Plant Growth Regul 19:195–216

Wallraff HG, Kiepenheuer J, Neumann MF, Streng A (1995) Homing experiments with starlings deprived of the sense of smell. The Condor 97:20–26

Wegener R, Schulz S, Meiners T, Hadwich K, Hilker M (2001) Analysis of volatiles induced by oviposition of elm leaf beetle Xanthogaleruca luteola on Ulmus minor. J Chem Ecol 27:499–515

Wu J, Baldwin IT (2009) Herbivory-induced signalling in plants: perception and action. Plant Cell Environ 32:1161–1174

Zangerl AR, Hamilton JG, Miller TJ, Crofts AR, Oxborough K, Berenbaum MR, de Lucia EH (2002) Impact of folivory on photosynthesis is greater than the sum of its holes. Proc Natl Acad Sci USA 99:1088–1091