ECOLOGY LETTERS

Ecology Letters, (2020) **23**: 1073–1084 doi: 10.1111/ele.13506

LETTER

Herbivory meets fungivory: insect herbivores feed on plant pathogenic fungi for their own benefit

Franziska Eberl,[1] Maite Fernandez de Bobadilla,[1,†] Michael Reichelt,[1] Almuth Hammerbacher,[2] Jonathan Gershenzon[1] and Sybille B. Unsicker[1]*

The peer review history for this article is available at https://publons.com/publon/10.1111/ele.13506

Abstract

Plants are regularly colonised by fungi and bacteria, but plant-inhabiting microbes are rarely considered in studies on plant–herbivore interactions. Here we show that young gypsy moth (*Lymantria dispar*) caterpillars prefer to feed on black poplar (*Populus nigra*) foliage infected by the rust fungus *Melampsora larici-populina* instead of uninfected control foliage, and selectively consume fungal spores. This consumption, also observed in a related lepidopteran species, is stimulated by the sugar alcohol mannitol, found in much higher concentration in fungal tissue and infected leaves than uninfected plant foliage. Gypsy moth larvae developed more rapidly on rust-infected leaves, which cannot be attributed to mannitol but rather to greater levels of total nitrogen, essential amino acids and B vitamins in fungal tissue and fungus-infected leaves. Herbivore consumption of fungi and other microbes may be much more widespread than commonly believed with important consequences for the ecology and evolution of plant–herbivore interactions.

Keywords

gypsy moth, mycophagy, nutritional ecology, rust fungus, Salicaceae, tripartite interaction.

Ecology Letters (2020) **23**: 1073–1084

INTRODUCTION

The co-evolution of herbivorous insects and their host plants has been intensively studied for decades (Futuyma & Agrawal 2009). However, plants are closely associated not only with insects but also with microbes (Baldrian 2017) that may also play a role in plant–herbivore interactions in favour of either the plant or the herbivore. The effect that plant-colonising microbes have on plant–herbivore interactions has been studied especially in grass–endophyte systems (Fernandez-Conradi *et al*. 2018). However, almost none of the work has disentangled the direct and indirect effects of microbes on herbivores.

Indirect effects of microorganisms on herbivores may involve microbe-induced changes in the plant that influence the behaviour or performance of the herbivore. For example, microbes can detoxify plant defence compounds (Hammerbacher *et al*. 2013; Mason *et al*. 2014) or enhance plant defences against herbivores (Van Wees *et al*. 2008). Furthermore, plant-inhabiting microbes may alter source–sink relationships in the plant (Berger *et al*. 2007) or modulate defence hormone signalling (Glazebrook 2005). Antagonistic hormone crosstalk during simultaneous pathogen and herbivore attack (Eberl *et al*. 2018), for example, may repress anti-herbivore defences such as toxin production or attraction of natural enemies (Desurmont *et al*. 2016).

The direct effects of plant-associated microbes on herbivores involve herbivore ingestion of microbes or microbial metabolites (Eberl *et al*. 2019). Despite the high frequency of such interactions in nature, we know little about whether insects ingest microbial tissue accidentally or intentionally and whether this affects their performance. Intentional fungivory, or mycophagy, has been described for many species, such as mites, beetles and gastropods (Oliveira *et al*. 2014; Sutherland & Parrella 2009; Ramsell & Paul 1990). Fungal tissue has a similar caloric value and macro-nutrient content as plant leaves, but lacks some typical defence compounds, and provides a rich source of B vitamins and sterols (Martin 1979).

Woody plant species can harbour an enormous diversity of insect and microbial species simultaneously due to their large dimensions, long life span and diverse niches (Lämke & Unsicker 2018). We therefore chose a woody plant–herbivore–pathogen system to study herbivore fungivory and how this affects herbivore performance. Our system consisted of a deciduous tree, the black poplar (*Populus nigra*), the pathogenic rust fungus *Melampsora larici-populina* and a tree-feeding insect, the gypsy moth (*Lymantria dispar*). All three species are native to Europe and occur in the same habitat. Gypsy moth larvae feed on a broad range of hosts from more than 40 plant families (Robinson *et al*. 2010), among them the Salicaceae, which includes poplar. Poplar trees are colonised by many different endophytes and pathogens (Mason *et al*. 2014; Busby *et al*. 2016), among the most devastating of which are the rust fungi (Pei & Shang 2005). In repetitive vegetative cycles on poplar leaves, rusts produce millions of uredospores (Hacquard *et al*. 2011), which then re-infect leaves of the same or neighbouring trees, leading to their frequent abundance in poplar stands.

[1]*Department of Biochemistry, Max Planck Institute for Chemical Ecology, Hans-Knöll-Straße 8, 07745, Jena, Germany*
[2]*Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private bag X20, Hatfield 0028, South Africa*
[†]*Wageningen University and Research Center, Laboratory of Entomology, Droevendaalsesteeg 1, 6708PB, Wageningen, The Netherlands*
**Correspondence: E-mail: sunsicker@ice.mpg.de*

In previous work, we demonstrated direct and indirect effects of rust infection on poplar-feeding gypsy moth. Specifically, caterpillars were attracted to rust spore volatiles and rust infection induced antagonistic hormone crosstalk in the host tree (Eberl *et al.* 2018). To place these results into an ecological context, we have now investigated the preference of gypsy moth larvae for rust-infected versus uninfected black poplar leaves and their performance on these two different food sources over a time course of rust and larval development. To allow more general conclusions, we also tested caterpillar feeding preference with another lepidopteran species, the rusty tussock moth, and with poplar leaves infected by mildew, another biotrophic plant pathogen. Additionally, we investigated the chemical basis of this behaviour by analysing plant and fungal tissues and examining the effects of single compounds on insect preference and performance. Our results revealed that phytopathogenic fungi positively influence gypsy moth preference due to their high content of a sugar alcohol and positively influence insect performance, likely due to the high level of amino acids in fungal tissue. With this, our study supports earlier hypotheses on the positive influence of fungivory (Hatcher 1995) and provides new levels of detail on the direct effects of plant-associated fungi on herbivores.

METHODS

Plants, insects and phytopathogens

Black poplar (*Populus nigra*) trees (1.5 m in height) were raised from stem cuttings of genotypes growing in a common garden in Germany (50°57'28.5" N 11°31'17.4" E). Caterpillar preference assays were performed with three different tree genotypes: chemical analysis, mannitol preference and performance assays were done with single clones. Cuttings were potted singly in 2-L pots in a 1:5 mixture of sand:soil (potting soil, Klasmann-Deilmann GmbH, Geeste, Germany) and grown in the greenhouse (18 °C/ 20 °C night/ day, humidity 60%). Experiments involving fungal infection were conducted in a climate chamber (18 °C/20 °C night/day, humidity 60%, 16 h light [MT 400, Eye, Uxbridge, UK]).

Gypsy moth (*Lymantria dispar* L.) caterpillars were reared as described in Eberl *et al.* (2018). Rusty tussock moth (*Orgyia antiqua*, L.) caterpillars were hatched from eggs of one female caught in the field.

Uredospores of the poplar leaf rust fungus (*Melampsora larici-populina* Kleb.) were obtained from naturally infected black poplar trees in the field. Fungal identity was verified as described (Eberl *et al.* 2018). Spores were amplified by infecting young trees and collecting uredospores 2–3 weeks post-infection. Spores were stored at −20 °C until the start of an experiment, either dried (preference assay) or fresh (other experiments). Trees were spray-inoculated with a spore–water mixture (dry: 1 mg ml^{-1}, fresh: 1.5 mg ml^{-1}) on the abaxial side of leaves (approximately 1 ml per leaf) and then covered with a polyethylene terephthalate bag (Bratschlauch, Toppits, Minden, Germany), which was kept closed for 1 day to ensure sufficient humidity. Control trees received the same treatments but with water only. Mildew (*Erysiphales spp.*) infection occurred irregularly in the greenhouse and thus time since infection was not determined.

Caterpillar preference and selective feeding

Caterpillar preference for rust, mildew, mannitol and catechin was tested with leaf discs, which were cut from mid-stem leaves (3rd to 10th leaf from apex) of different treatment groups and offered to individual second instar caterpillars ($n = 20$ for rust; $n = 26$ for mildew; $n = 20$ for mannitol and catechin) in custom-made Petri dishes (Fig. 1a; for details, see Boeckler *et al.* 2014). When using more than one tree genotype, genotypes were not mixed within one dish to minimise variability. After 2 days, the remaining leaf tissue was photographed and herbivory was determined using Adobe Photoshop CS5 (San Jose, CA, USA). After the mildew-preference assay, infected and non-infected discs were pooled separately for mannitol analysis.

To examine possible effects from cutting out leaf discs, the assay was repeated with attached, undamaged leaves (for details, see Fig. S1A).

In the rust choice assay, leaves from uninfected and rust-infected trees were used 11 days post-infection (dpi) or 12 dpi (gypsy moth or rusty tussock moth, respectively). However, the preference was also assessed at different time points of rust infection (for details, see Fig. S2) and for different instars and diet experience of the caterpillars (for details, see Table S1).

Leaves in the mannitol and catechin choice assays were coated with a thin layer (2.5 ml per 100 cm^2 leaf area) of 1.5% plant agar (Duchefa Biochemie, Haarlem, The Netherlands) containing 0.2 mg ml^{-1} D-mannitol (Roth, Karlsruhe, Germany) or 0.4 mg ml^{-1} catechin (Sigma-Aldrich, St. Louis, MO, USA) which corresponds to an increase of 0.75 or 1.5 mg g^{-1} dry weight (DW), respectively, similar to the observed increases in leaves upon rust infection. Control leaves received the same agar coating but without supplementation.

Selective feeding on fungal spores was quantified by keeping first instar *L. dispar* caterpillars for 72 h individually ($n = 9$) in Petri dishes containing one rust-infected poplar leaf (11 dpi). Rust sporangia were counted and caterpillar sporangia consumption was visually monitored over time. No new sporangia appeared during the experiments. The first occurrence of leaf damage was documented.

Caterpillar performance assay and tissue collection

To monitor gypsy moth performance, larvae ($n = 8$–9 for males; $n = 11$ for females) were transferred to rust-infected or uninfected poplar trees 2 days after hatching until pupation. Each larva was kept in a single leaf box (Fig. S3). At 3-day intervals, larvae were weighed and switched to a new food tree. The trees were inoculated with rust fungus (or water for controls) 7 days before being offered to the larvae. The pupae were kept at room temperature until emergence of the adults for sexing. Leaves from three rust-infected (10 dpi) and three uninfected control trees were harvested whole and flash-frozen 6 (Fig. 4), 12 and 18 days (Table S7) after the start of the performance experiment so that caterpillars had fed on each of the leaves for 3 days. Rust spores were collected from

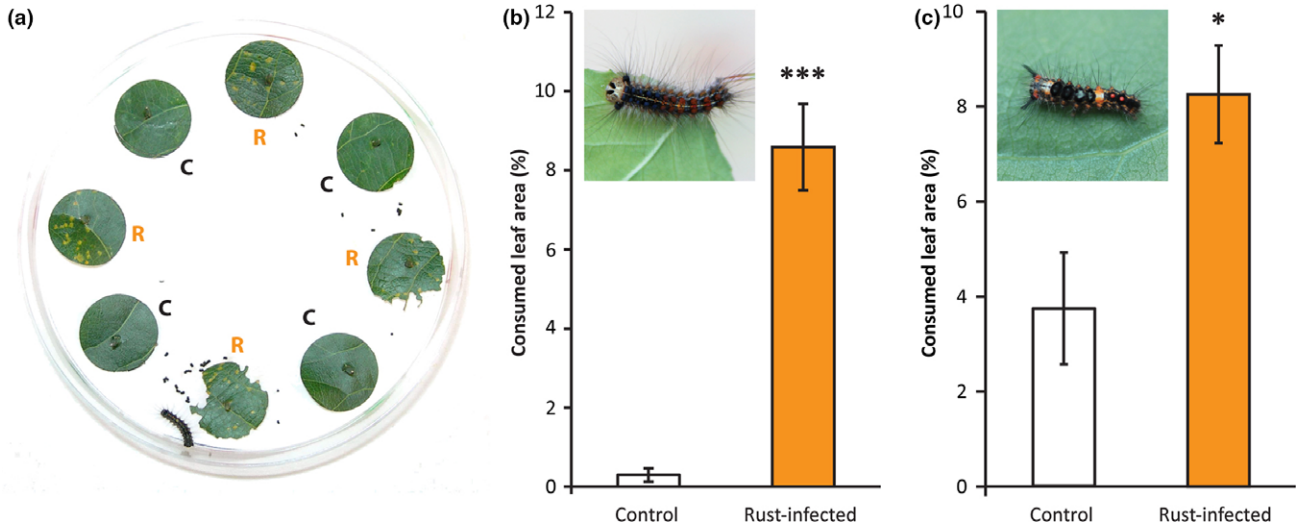

Figure 1 Feeding preference of lepidopteran larvae for rust-infected leaves tested in a choice assay (a). Larvae (2nd instar) of gypsy moth (*L. dispar*, b) and rusty tussock moth (*O. antiqua*, c) were allowed to feed on leaf discs of rust-infected (11–12 dpi, filled bars; 'R') and uninfected (empty bars, 'C') black poplar leaves. Preference was evaluated as % consumed area of total leaf area. Mean ± SEM (n = 20), Wilcoxon signed-rank test, $*P < 0.05$; $***P < 0.001$.

undamaged, artificially infected trees that were not used in the performance assay.

Tissues (leaves, mycelium) from mildewed and control poplars (see Fig. S4) were harvested from undamaged trees of the same genotype.

Chemical analyses

Leaves, mildew mycelium and rust spores were lyophilised. Leaf powder (10 mg) and mycelium (6 mg) were extracted with methanol (0.1 mL per mg) containing 0.8 mg ml^{-1} phenyl-β-glucoside (Sigma-Aldrich) as internal standard by shaking twice in a paint shaker for 30 s each. Rust spores (5–6 mg) were extracted with 0.5 ml of the same solvent (each in two to three technical replicates) in aluminium tubes with steel beads by shaking 3 × 5 min to break the spore wall. Spores present on leaves were not extracted by our standard leaf extraction protocol.

Phenolic compounds (salicinoids, flavonoids) in the extracts were analysed by HPLC-UV on a reversed phase column (Nucleodur Sphinx, RP 5 µm, Machery-Nagel, Düren, Germany), after 1:2 dilution of the extract with water, as described previously (Boeckler *et al.* 2013). The analytes were quantified relative to the peak area of the internal standard (see Table S2).

Amino acids were quantified by LC-MS/MS (for details, see Crocoll *et al.* 2016) on a C18-column (XDB-C18; Agilent, Santa Clara, CA, USA) after diluting the extract 1:10 with water containing 10 µg ml^{-1} of a mix of $^{15}N/^{13}C$ labelled amino acids (Isotec, Miamisburg, OH, USA). The amino acids were quantified relative to the peak area of their corresponding labelled amino acids, except for tryptophan (relative to phenylalanine, response factor 0.42) and asparagine (relative to aspartate, response factor 1.0).

Soluble sugars and mannitol were analysed from the extract (at 1:10 dilution in water), by LC-MS/MS on a hydrophilic interaction liquid chromatography (HILIC) column (apHera-NH_2 Polymer; Supelco, Bellefonte, PA, USA) as described in Madsen *et al.* (2015) or in Table S3, respectively. All analytes were quantified using an external standard curve with authentic standards of glucose, fructose, sucrose, stachyose (all from Sigma-Aldrich), raffinose (Fluka, Seelze, Germany) and mannitol (Roth).

Nitrogen content was analysed from 8 to 15 mg dried leaf tissues and spores, with a varioEL elemental analyzer (Elementar Analysensysteme, Langenselbold, Germany).

B vitamins were analysed from the extract by LC-MS/MS using a method described by Khaksari et.al. (2018) with modifications (Table S3).

Statistics

All data were tested for statistical assumptions, that is, normal distribution and homogeneity of variances. Whenever necessary, data were log-transformed. For caterpillar preference data, either paired *t*-tests or Wilcoxon signed-rank tests were performed. Data on caterpillar performance were evaluated by repeated measures ANOVA. Chemical analysis data were either tested with Student's *t*-test, ANOVA or Welch-ANOVA, plus Tukey's or Games–Howell *post hoc* test, depending on the homogeneity of variances, or Kruskal–Wallis test, in case of non-normal distribution. All statistical analyses were performed with SPSS 17.0 (SPSS, Chicago, IL, USA).

RESULTS

Lepidopteran larvae prefer pathogen-infected leaves

In order to determine the preference of caterpillars towards pathogen-infected *versus* uninfected plant tissue, we conducted preference assays with black poplar leaves.

In a two-choice assay with rust-infected and uninfected control leaves, second instar gypsy moth caterpillars consumed almost exclusively the rust-infected leaf discs and showed the same preference when whole leaves were still attached to the tree (Fig. 1b and S1B; Wilcoxon signed-rank test, $P < 0.001$ and $P = 0.014$, respectively). To test the generality of this observation, we also studied a closely related species, the rusty tussock moth. Larvae consumed about twice as much from the rust-infected as from the uninfected leaf discs (Fig. 1c; Wilcoxon signed-rank test, $P = 0.019$). During experiments on both insects, we observed selective feeding on sporangia of the rust fungus (Fig. S3; Video). Over a time period of 72 h, nearly all caterpillars first consumed the sporangia present before they started feeding on leaf material (Fig. 2).

In order to study the specificity of insect preference for pathogen-infected leaves, we also conducted a preference assay with mildew-infected black poplar foliage. Here, gypsy moth larvae also clearly preferred infected leaves, consuming approximately three times as much area from mildew-infected as from uninfected leaves (Fig. 3b; Wilcoxon signed-rank test, $P = 0.001$). Interestingly, larvae also first abraded the fungal mycelium on the surface of infected leaves (Fig. 3a) before they consumed the leaf matrix.

When testing the feeding preference of differently aged caterpillars, we found that gypsy moth larvae lose their preference for rust-infected leaves at later instars (Table S1). While first and second instars preferred feeding on rust-infected leaf discs over uninfected control discs (1st instar: Wilcoxon signed-rank test, $P = 0.056$; 2nd instar: paired t-test, $P < 0.001$), no trend was visible for third instar larvae (Wilcoxon signed-rank test, $P = 0.657$). The feeding experience of young caterpillars, however, did not influence their preference, as both artificial diet-reared and poplar-reared gypsy moth larvae consumed significantly more rust-infected than uninfected leaf area (Table S1; diet-reared: paired t-test, $P < 0.001$; poplar-reared: Wilcoxon signed-rank test, $P = 0.005$).

Besides larval stage, caterpillar preference also depended on time course of fungal infection (Fig. S2). One day post-infection (dpi), larvae preferred uninfected leaves over rust-infected

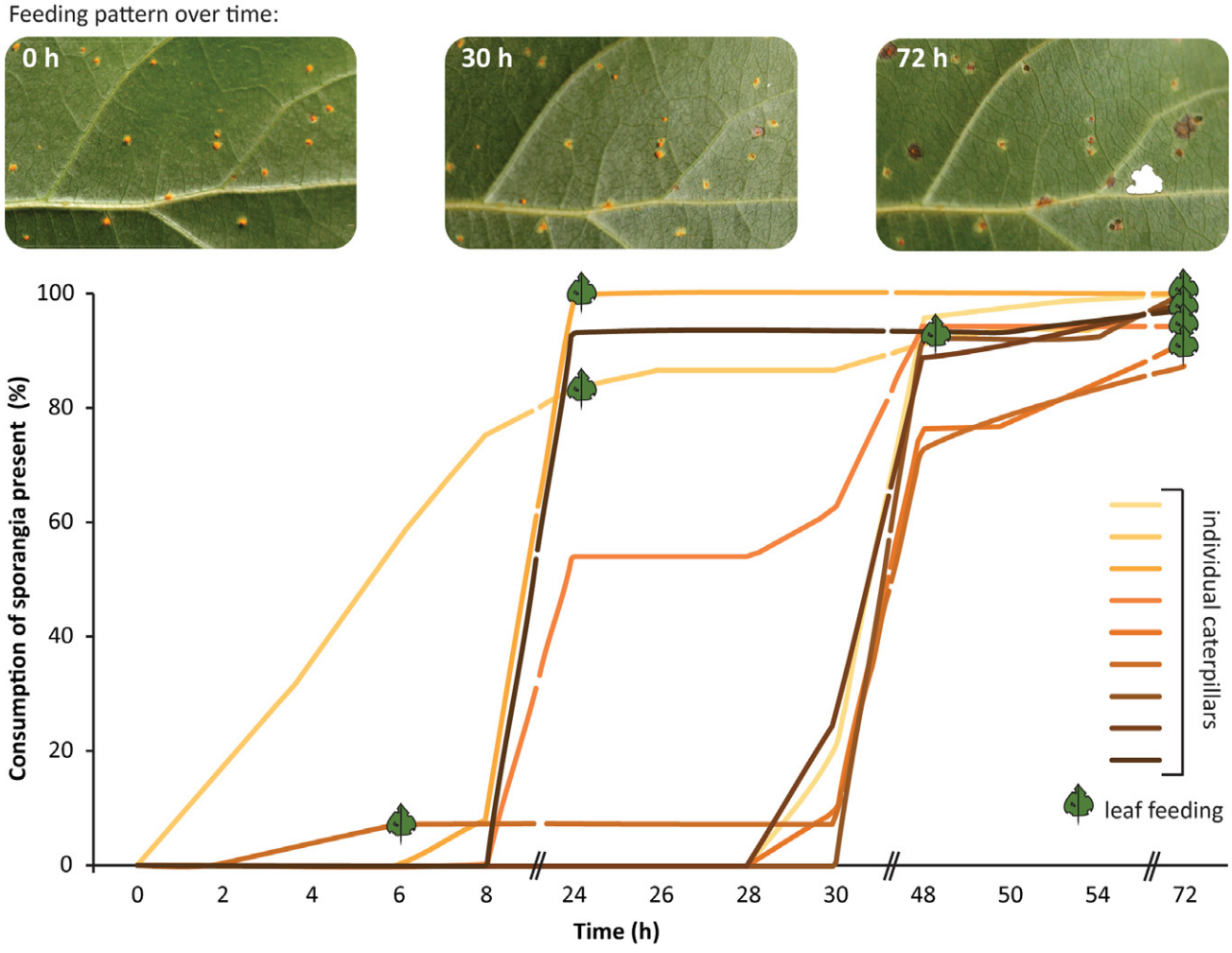

Figure 2 Selective feeding of young gypsy moth larvae on rust fungus sporangia. First instar larvae (moulted to 2nd instar during experiment) were observed individually for 72 h on rust-infected black poplar leaves. Consumption of sporangia was evaluated over time based on the percentage of total sporangia remaining on each leaf. The first occurrence of leaf feeding was also documented (leaf symbol). Representative photographs of the feeding pattern are shown for the beginning of the experiment (0 h), the stage of sporangia feeding (30 h) and the first occurrence of leaf feeding (72 h).

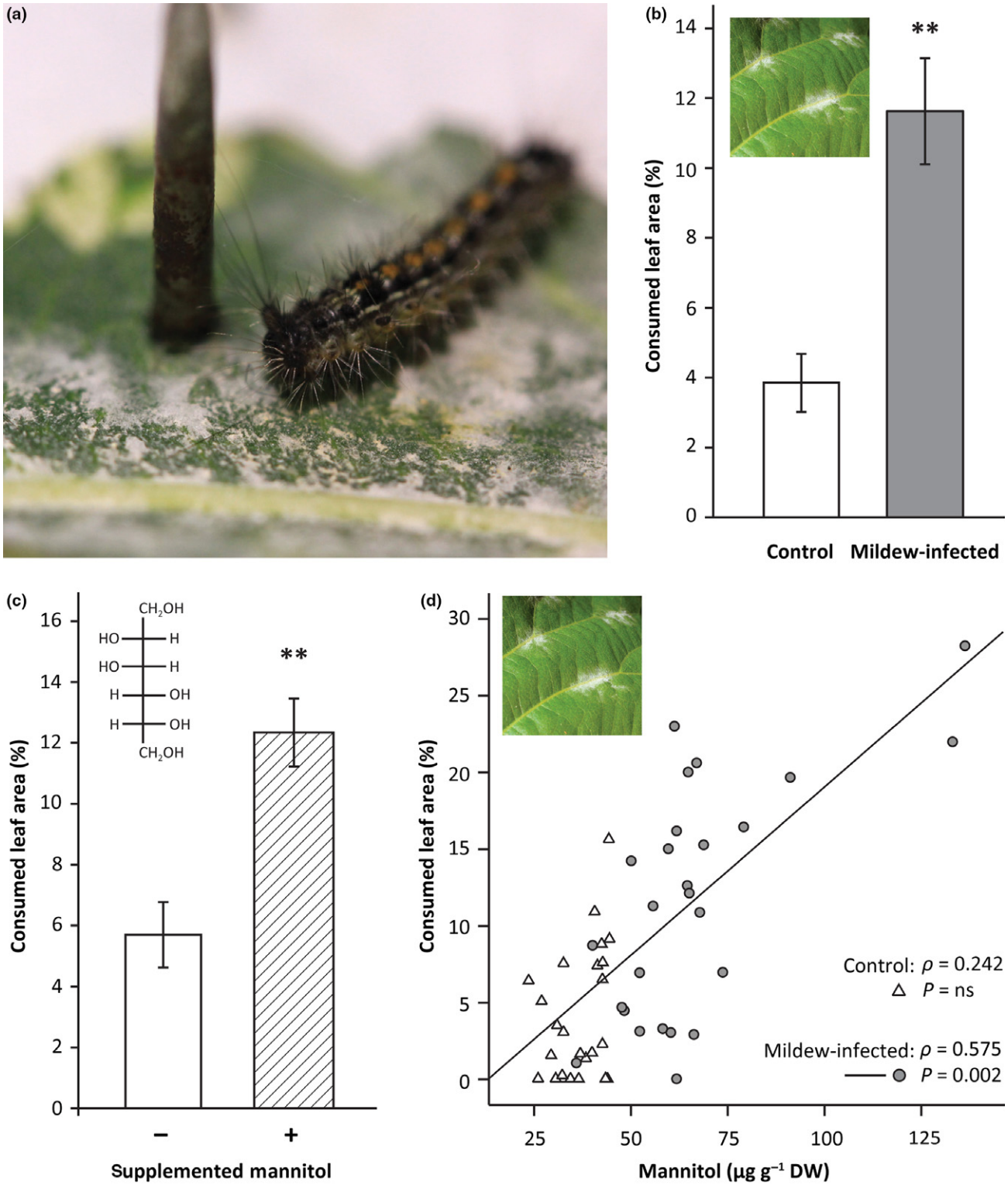

Figure 3 Feeding preference of gypsy moth larvae for mildew-infected leaves and mannitol. Caterpillars (2nd instar) selectively grazed on fungal mycelium present on the surface of mildew-infected black poplar leaves (a). For the preference assay, they were allowed to feed on leaf discs from uninfected (empty bar) and mildew-infected (filled bar) black poplar leaves for 2 days (b). To test for mannitol preference, leaves were coated with plant agar with (striped bar; +) or without (empty bar; −) supplemented mannitol (0.2 mg ml^{-1}; c). Feeding damage in the mildew-preference assay (see a) correlated with mannitol content in mildew-infected but not in control leaf discs (d). Preference was recorded as percent consumed of total leaf area. Mean ± SEM (n = 26 (b and d); n = 20 (c)), paired t-test (a), Wilcoxon signed-rank test (c): $^{**}P < 0.01$; Spearman's rank correlation (d).

leaves (paired *t*-test, $P = 0.009$), but preference switched at 4 dpi towards rust-infected leaf discs (paired *t*-test, $P < 0.001$). This preference remained through the rest of the experiment, and became strongest at the last time point (10 dpi) with 78% of the consumed leaf area being rust-infected (Wilcoxon signed-rank test, $P < 0.001$).

Fungal tissue contains high amounts of sugar alcohol

In order to identify the trait(s) responsible for this feeding preference, pathogen-infected and uninfected black poplar leaves, as well as fungal tissues, were chemically characterised. We analysed various classes of compounds that might be deterrent or nutritionally relevant.

Levels of salicinoids, the characteristic defence compounds of the Salicaceae family (Boeckler *et al.* 2011), were not significantly different between rust-infected and uninfected control leaves, but were rarely present in the rust spores (Fig. 4a; ANOVA: $F = 101.7$, $P < 0.001$). Mildew infection led to a significant decrease in salicinoids in poplar leaves, and mildew mycelium contained almost 100 times less of these compounds than plant leaves (Fig. S4A; ANOVA: $F = 92.8$, $P < 0.001$). Flavonoids, another group of phenolic defence compounds, which includes catechin, increased in leaves after rust infection ($t = -4.4$; $P = 0.012$), but were not detected in fungal spores and only in low amounts in mildew mycelium (Fig. 4b, S4B; ANOVA mildew: $F = 59.8$, $P < 0.001$). Free amino acids were not significantly different between rust- or mildew-infected and uninfected leaves, but were considerably higher in the fungal compared to plant tissues (Fig. 4c, Fig. S4C; ANOVA, rust: $F = 29.8$, $P = 0.001$; mildew: $F = 44.0$, $P < 0.001$). Nitrogen content did not differ among different groups of poplar leaves, but was elevated in rust spores (Fig. 4d; $F = 33.8$, $P < 0.001$). Levels of soluble sugars, mainly sucrose, did not change in black poplar leaves upon rust or mildew infection (Fig. 4e; Fig. S4E). In both fungal tissues, soluble sugars were present in substantially lower amounts compared to the leaves, leading to a significant difference among the different tissues (ANOVA, rust: $F = 164.4$, $P < 0.001$; mildew: $F = 93.8$, $P < 0.001$). We also analysed mannitol, a sugar alcohol known to be produced by some plant pathogens (Jennings *et al.* 1998; Voegele *et al.* 2005), and observed striking differences between plant and fungal tissues (ANOVA, rust: $F = 164.4$, $P < 0.001$; mildew: $F = 183.3$, $P < 0.001$). Mannitol concentration increased in poplar leaves following rust or mildew infection. Furthermore, the mannitol concentration found in rust spores was 20 and 400 times greater than in rust-infected and control leaves, respectively (Fig. 4F), and mildew mycelium reached mannitol concentrations about 50–60 times higher than leaves (Fig. S4f). Additional analyses of leaf tissues and rust spores supported these general patterns (Table S7).

To test whether mannitol stimulates the feeding of gypsy moth larvae, we coated black poplar leaves with a thin layer of agar containing mannitol. In a choice assay, young caterpillars consumed significantly more of the mannitol-supplemented discs compared to discs without mannitol (Fig. 3c; paired *t*-test: $P = 0.001$). We also analysed mannitol levels in leaf discs after the mildew-preference assay (Fig. 3b) and found a strong positive correlation between the leaf area consumed by gypsy moth larvae and the concentration of mannitol in mildew-infected leaf discs (Fig. 3d). The flavonoid catechin, however, which was also elevated in rust-infected poplar leaves (Fig. 4b), did not influence caterpillar preference (Fig. S6).

Gypsy moth larvae develop faster on pathogen-infected leaves

After observing the clear feeding preference of gypsy moth larvae for rust-infected poplar leaves, the fitness consequence of this choice was investigated. Gypsy moth larvae were reared on rust-infected or uninfected control black poplar foliage from the first instar to pupation and were weighed regularly.

Overall, caterpillars that were reared on rust-infected trees gained significantly more weight over time than those reared on uninfected controls (Fig. 5a; two-way ANOVA: factor 'Rust', $P < 0.001$). By the last time point of monitoring, the day of the first pupation (21 days), larvae on rust-infected trees weighed twice as much as their conspecifics reared on uninfected control trees. However, pupal and adult weight did not differ between the two treatments (Fig. 5c and d). The increase in larval weight can be explained by an accelerated development of the larvae, as caterpillars on rust-infected trees pupated 3 (♂) to 4 (♀) days earlier than those in the control group (Fig. 5b), which represents ca. 10 % of the larval development time.

To test whether mannitol influences their performance, we reared young gypsy moth larvae *in vitro* on leaves with or without mannitol supplementation. There was, however, no difference in larval weight between the caterpillars feeding on mannitol-supplemented or control leaves (Table S4).

Free amino acids were found to be present in higher concentrations in the rust spores compared to leaf tissues (Figs 4c, 6). Comparing amino acids essential for insects, fungal spores had the highest abundance (Table S5), with levels of Leu, Met, Thr, Arg and His being significantly different among tissues, lowest in control leaves and highest in fungal spores (Table S5).

Additionally, leaves infected with rust as well as rust spores alone showed higher levels of some B vitamins than uninfected leaves (Table S6). Nicotinic acid (vitamin B3) and panthothenic acid (vitamin B5) were significantly elevated in both spores and rust-infected tissue compared to control leaves. Biotin (vitamin B7) was significantly more concentrated in spores compared to uninfected poplar leaves, and rust-infected leaves showed a strong positive trend to having higher concentrations than uninfected leaves. Interestingly, spores did not accumulate riboflavin (vitamin B2) and contained lower amounts of this vitamin compared to leaves.

DISCUSSION

Since fungi and other microbes commonly colonise plant tissues, many herbivores may also be inadvertent fungivores. Here we report a case of deliberate fungivory by herbivores. First and second instar lepidopteran larvae given black poplar leaves feed selectively on sporangia and mycelium of plant

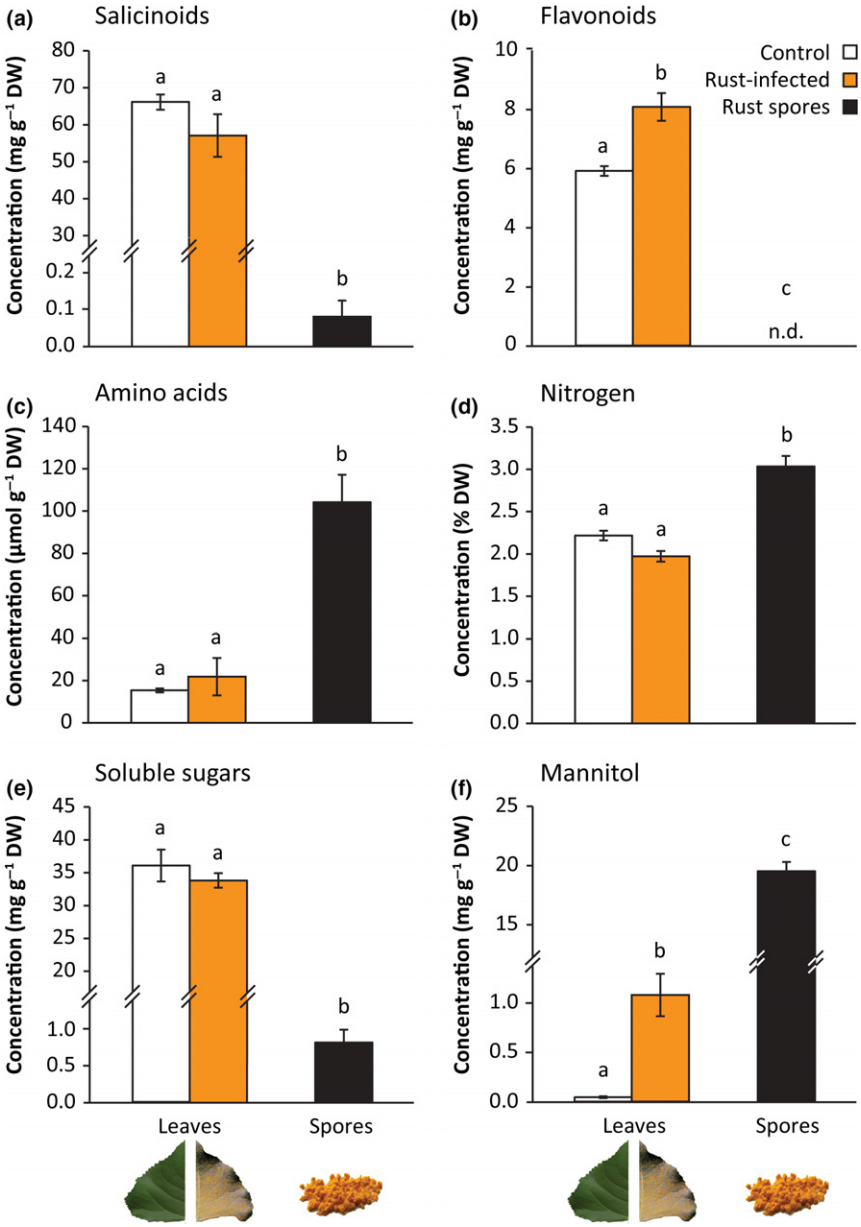

Figure 4 Chemical analysis of plant leaves and fungal spores. Rust-infected (orange bars) and uninfected control (empty bars) black poplar leaves as well as separated uredospores of the poplar leaf rust fungus (black bars) were chemically characterised. Presented are data for salicinoids (a; sum of salicin, salicortin, homaloside d, 6'-*O*-benzoylsalicortin), flavonoids (b; sum of catechin, proanthocyanidin B1, rutin), amino acids (c; sum of free amino acids), nitrogen content (d), soluble sugars (e; sum of glucose, fructose, sucrose, tri- and tetrasaccharides) and the sugar alcohol mannitol (f). Mean ± SEM (n = 3); ANOVA with *post-hoc* test, different letters indicate significant differences among groups; n.d. = not detected. See also Table S7 for chemical analysis of poplar leaves harvested at other time points during the experiment and additional spore samples.

pathogenic fungi and prefer pathogen-infected leaves to uninfected controls. We found that fungal feeding is characteristic of young larvae, employs mannitol as a feeding cue and speeds larval development. The latter may be related to higher levels of total nitrogen, essential amino acids and B vitamins.

Generalist herbivores feed on fungal tissue

Young gypsy moth larvae preferred feeding on rust-infected over uninfected poplar leaves and fed selectively on fungal sporangia on the surface of infected leaves. The rusty tussock moth, a close relative of the gypsy moth, likewise preferred rust-infected leaves and sporangia. Fungivory in Lepidoptera is rarely studied and has been reported in just a few families, such as the Tineidae (Rawlings 1984). In the Erebidae and Noctuidae, a few observations on fungus-feeding individuals were recorded (Yoshimatsu & Nakata 2006; Moskowitz & Haramaty 2012), but systematic studies are lacking. The fungus-feeding behaviour we observed in two erebid species suggests that facultative fungivory is more common than

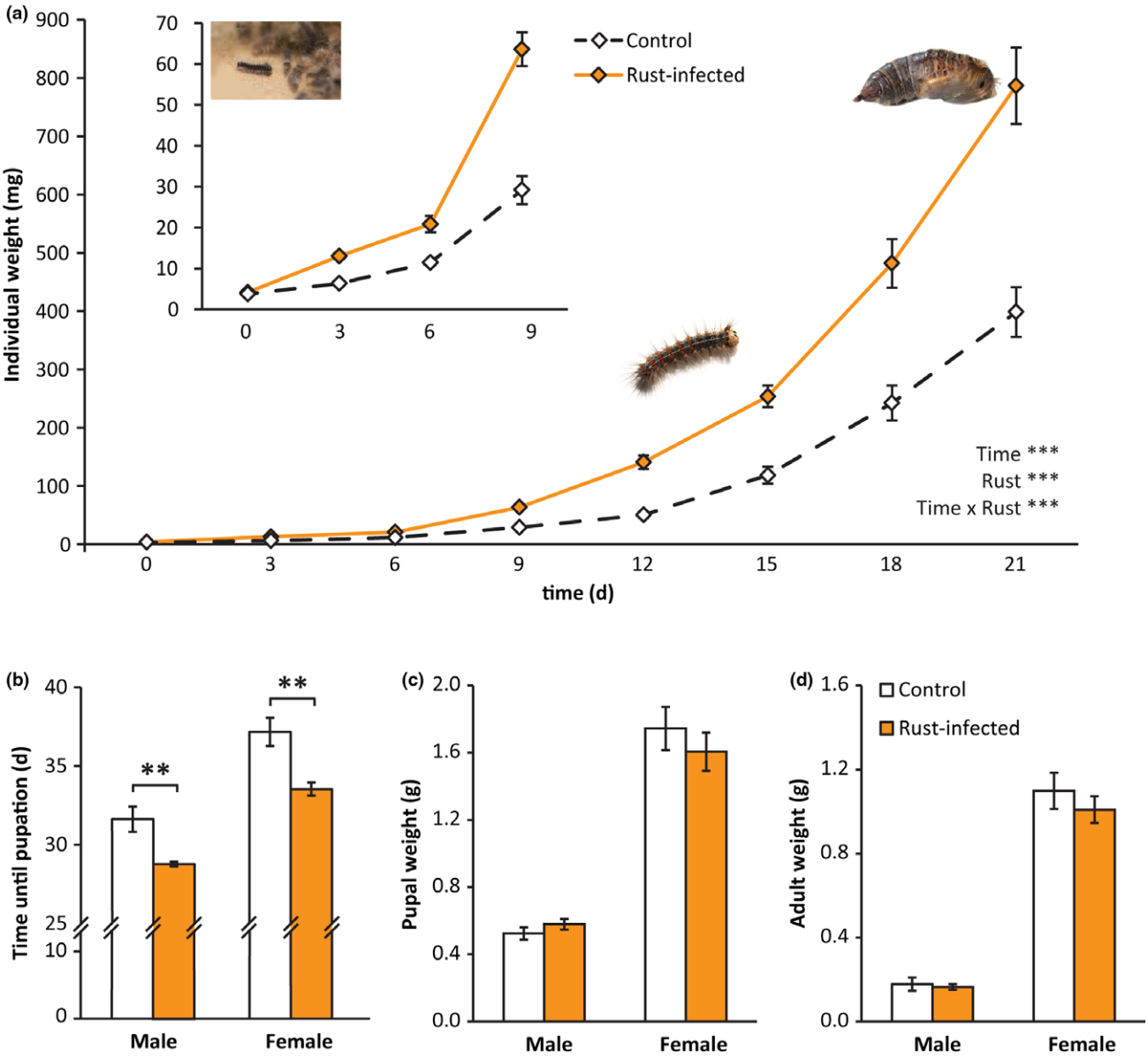

Figure 5 Performance of gypsy moth larvae on rust-infected versus uninfected control leaves. Caterpillars were fed on rust-infected black poplar trees (7–10 dpi, leaves with fungal spores present; filled symbols, solid line) or uninfected controls (empty symbols, dashed line) and were weighed every 3 days (a). Data are shown from the onset of the experiment (0 d, i.e. 2 days after hatching) until the beginning of the first pupation (21 days). The inset shows an expanded scale for early time points (0–9 days). Results from repeated measures ANOVA are given for the factors time, infection and their interaction (n = 19–20; ***P> 0.001). Larval developmental time (b), pupal (c) and adult weight (d) are shown for male and female gypsy moths after rearing on rust-infected and uninfected control trees. Mean ± SEM (n = 8 [control] and 9 [rust-infected] for male; n = 11 for female), Mann–Whitney U-test (time) or Student's t-test (weights); significant differences are marked with asterisks (**P < 0.01).

previously assumed. In the past, the actual extent of facultative fungivory among herbivores was difficult to estimate, since the separation of fungal and plant material is difficult under natural conditions. Modern molecular techniques, however, now allow qualitative and quantitative analyses of microbial content.

Gypsy moth caterpillar preference for fungal tissue was not found to be fungus species-specific as caterpillars also preferred leaves infected with powdery mildew, another biotrophic pathogen, as well as its surface mycelium. Since gypsy moth caterpillars are generalist feeders and mildew also colonises numerous plant species, it is very likely that such tripartite interactions also occur on other hosts. A preference for pathogen-infected plant tissue has so far been reported only anecdotally for some arthropod species, such as other lepidopteran species (Mondy *et al.* 1998; Rizvi *et al.* 2015), aphids (Johnson *et al.* 2003), earwigs (Barbe 1964), thrips (Yarwood 1943) and mites (Reding *et al.* 2001).

Caterpillar preference in our study depended on the time course of infection, changing from preference for uninfected leaves in the beginning to strong preference for rust-infected leaves at later time points. The increasing preference for

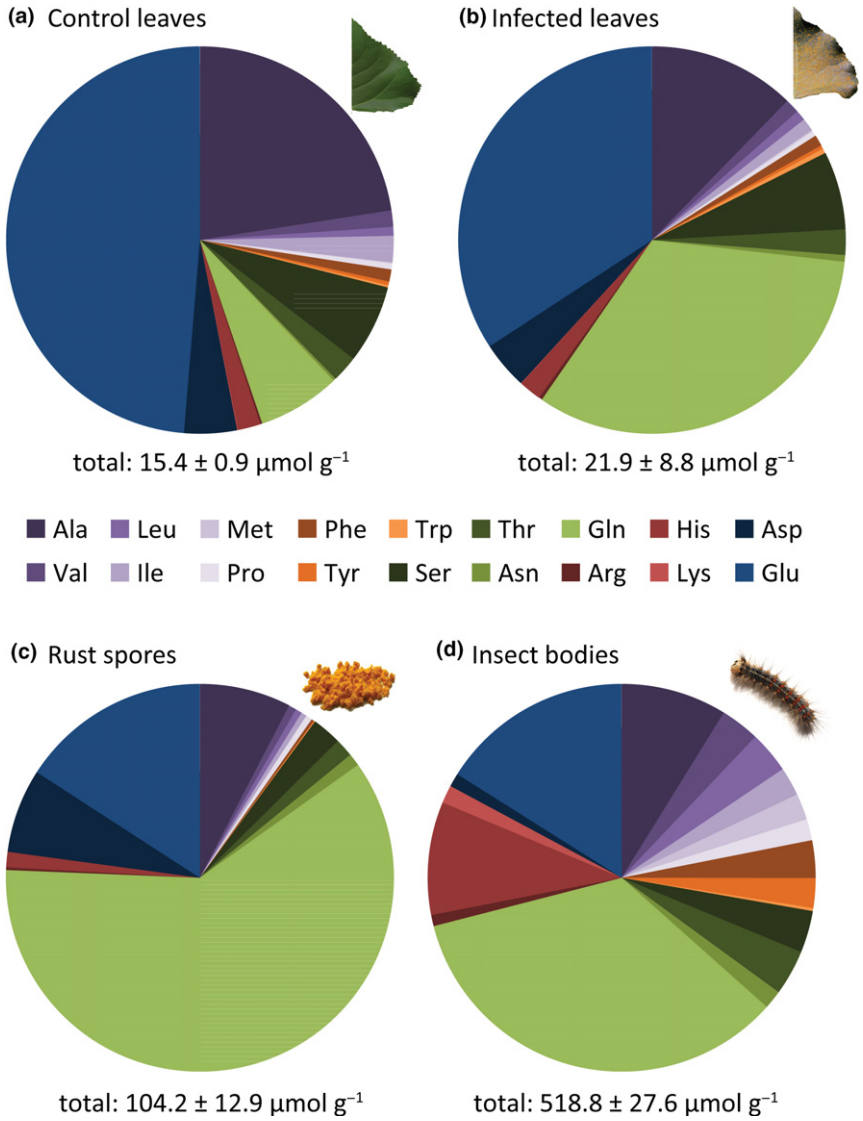

Figure 6 Composition of free amino acids in poplar leaves, fungal spores and caterpillar bodies. Leaves of black poplar were either uninfected (a) or rust-infected (10 dpi, leaves with sporangia already present, b). Uredospores of *Melampsora larici-populina* were separated from leaf material prior to analysis (c). Gypsy moth caterpillar bodies (4th instar) were analysed after the larvae had fed for 13 days on black poplar leaves (d). Shown are results for caterpillars that fed on uninfected leaves, which did not differ from those that fed on rust-infected leaves. Total amount of amino acids per dry weight is given below the charts as mean ± SEM ($n = 3$ for leaves, spores; $n = 10$ for insects). Amino acids are sorted by type of side chain: aliphatic (purple), aromatic (orange), polar (green), positively charged (red), negatively charged (blue).

infected tissue at later stages of infection might be due to more dense colonisation by fungal hyphae and a higher concentration of fungus-specific compounds, as well as the emergence of sporangia that were especially preferred by the larvae. The preference shift might also be based on temporal changes in the phytohormones jasmonic acid and salicylic acid during rust infection (Ullah *et al.* 2019), which could induce or suppress anti-herbivore defences, respectively.

Furthermore, the ontogeny of the insect influenced its preference. First and second instar caterpillars strongly preferred rust-infected leaves and also selectively fed on fungal material, which is in contrast to third instar larvae that showed no preference. The food preference of herbivorous insects is known to change during development (Unsicker *et al.* 2008), and early instars usually discriminate more strongly among different food sources than late instars (Browne 1995). This trend might originate from the fact that young instars are more vulnerable to parasitism, virus infections, starvation, weather effects and phytotoxins (Elkinton & Liebhold 1990; Zalucki *et al.* 2002).

Whether or not fungi benefit from this interaction with insects has not been investigated thoroughly. However, rust spores that had passed through caterpillar digestive tracts were no longer viable (Fig. S7), and therefore it seems unlikely that insect ingestion would increase dispersal.

Mannitol contributes to feeding preference of caterpillars

Previous work on gypsy moth demonstrated that caterpillars are attracted to pathogen-infected poplars by means of

olfaction (Eberl *et al*. 2018). Here we showed a feeding preference towards fungus-infected leaves and fungal tissues. All tissues were analysed to get an indication of the chemicals responsible for this attraction. Of the compounds measured, the correlation of mannitol with caterpillar feeding preference was especially strong. This sugar alcohol increased in rust- as well as mildew-infected leaves compared to controls and accumulated to even higher levels in fungal spores and mycelium that were especially preferred by caterpillars. Poplar leaves supplemented with mannitol were also preferred by young gypsy moth caterpillars over non-supplemented leaves, indicating a phagostimulatory effect. Furthermore, the mannitol content in mildewed leaves strongly correlated with feeding damage by gypsy moth caterpillars.

The few studies that have investigated the response of insects to mannitol report deterrent (Akeson *et al*. 1970), neutral (Schiff *et al*. 1989) or phagostimulatory (Takada *et al*. 2017) effects of this compound. This variable response suggests that the preference for mannitol might be adaptive for only some herbivores under specific conditions, rather than being a general cue, for example, for nutrients. For gypsy moth, mannitol might be an indication of fungal infection of the host plant, which provides good conditions for larval development.

However, the caterpillar preference for fungal tissue and fungus-infected foliage could also be explained by other variables, such as the low amounts of salicinoids. These phenolic compounds presumably deter gypsy moth larvae (Boeckler *et al*. 2014). Additionally, the high amounts of free amino acids and B vitamins in the fungal material might promote caterpillar fungivory.

Fungivory accelerates caterpillar development

In a performance experiment, we investigated the consequence of food preference for caterpillar fitness. Gypsy moth caterpillars reared on rust-infected black poplars gained twice as much weight as individuals reared on uninfected foliage. The difference in weight gain among the groups appeared in the first and second instars and continued throughout development. During these early instars, larvae on rust-infected trees primarily fed on the sporangia, indicating that the fungal diet provided more nutrition to the insects than leaves. This led to a faster development of rust-reared caterpillars in the early instars as compared to the control group. Consequently, larvae feeding on rust-infected leaves required three (male) to four (female) days less to pupate than their conspecifics on uninfected trees. Shortening the larval development time is an important fitness benefit, as it reduces the exposure to natural enemies and unfavourable environmental conditions, and might be advantageous in feeding competition as well (Benrey & Denno 1997). Accelerated development after feeding on pathogen-infected tissue was also observed in *Ostrinia nubilis* feeding on maize (Carruthers *et al*. 1986). But, in general, the effects of plant–pathogen interactions on insect fitness are very diverse (Hatcher 1995; Shikano *et al*. 2017; Eberl *et al*. 2019).

But what chemical trait could mediate the benefit for the insects? Mannitol, which accumulated in high concentrations in the fungal spores, did not influence larval performance when supplemented on poplar leaves. This suggests that mannitol does not have any nutritional value for gypsy moth larvae. However, the virtual absence of salicinoids in the fungi might increase the growth of young larvae that feed on spores instead of poplar leaves, which are rich in these anti-herbivore compounds (Boeckler *et al*. 2016).

Total nitrogen was significantly higher in rust spores than in poplar leaves. Given the importance of nitrogen in insect development (Scriber & Slansky 1981; Lindroth *et al*. 1997), we hypothesise that this factor is highly relevant for improved caterpillar performance on rust-infected poplars. Plant tissue is well known to represent a suboptimal nitrogen source for herbivorous insects due to both its low absolute amount (Martin & Kukor 1984) and low availability during digestion (White 1993). Rust spores, however, contained approximately 50 % more total nitrogen than plant tissue, reaching a level of 3%, which corresponds to the nitrogen intake target of gypsy moth larvae as measured in artificial diets (Stockhoff *et al*. 1993). In other systems, rust infection led to elevated nitrogen content in the host plants (Reddy & Rao 1976; Ramsell & Paul 1990; Al-Naemi & Hatcher 2013), which also positively correlated with the fitness of the herbivore studied (Al-Naemi & Hatcher 2013). In our study, mildew mycelium as well as rust additionally had about three- to five-fold elevated levels of free amino acids compared to plant tissues, respectively. Five of the essential amino acids were significantly higher in rust spores and rust-infected leaves compared to uninfected leaves. Among these amino acids are histidine, methionine and arginine, which are usually less abundant in plant protein and are therefore limiting for herbivore nutrition (Barbehenn *et al*. 2012). Furthermore, we found that the amino acid composition of rust-infected leaves as well as that of mildew mycelium resembled the composition of caterpillar bodies more closely than uninfected leaves did, which possibly lowers the costs for biochemical conversion for larvae feeding on these diets.

Another important dietary requirement for insects are vitamins (Friend 1958), among them the B vitamins that are present at high levels in fungi in general (Martin 1979). In our study system, we found that fungal infection leads to an increase in certain B vitamins in the plant leaves as well as high levels in the fungal tissue itself. The elevated amounts of these essential micronutrients might help accelerate caterpillar growth and development.

Apart from the traits listed above, other characteristics of fungal tissue might better support insect performance in comparison to plant tissue. For example, the absence of certain typical plant metabolites in fungi, such as condensed tannins or lignin (Martin 1979; Hatcher 1995), could improve the rate of ingestion and digestion for insects. Lower tissue toughness may also benefit insects, especially those with small or weak mandibles. Furthermore, fungi may have higher levels of certain minerals and polyunsaturated fatty acids than plants (Martin 1979). Given the many reports of how microbes supply critical nutrients for insects (Douglas 2015), such fungivory on plant pathogens or non-pathogenic endophytes may even be essential for certain insects. The use of sterile plants would facilitate investigations of how plant-associated fungi contribute to insect nutrition.

CONCLUSIONS

The feeding preference of insect herbivores for fungus-infected foliage may be much more common than previously recognised, but more detailed surveys are needed to systematically detect and characterise microbes in the food sources of herbivores. Evidence for widespread fungivory by insect herbivores may have important consequences for studies of plant–insect interactions and co-evolutionary theories (Biere & Tack 2013). Herbivore host seeking behaviour may be altered if fungi are a primary food source. Plant defence strategies may be modified to protect more strongly against fungal invasion, if phytopathogenic infections favour the development of herbivores. And, fungi themselves may have developed traits to attract or deter herbivores, depending on whether or not they profit from their association with herbivores. Considering these aspects of microbial involvement will add new perspectives on plant–herbivore interactions.

ACKNOWLEDGEMENT

We thank Ines Hilke from the MPI for Biogeochemistry for the elemental analysis, Beate Rothe and the student helpers Robert Lotze and Sindy Arnreich for assisting during the experiments, Hannah Nadel for providing gypsy moth eggs, Sandra Lackner for helpful discussions, Daniel Veit for constructing the performance assay setup, Emily Puckett for assisting in the development of B vitamin analysis, and the gardeners of the MPI for Chemical Ecology for rearing plants. This study was funded by the Max Planck Society.

COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

FE, SU, AH and JG conceived the project; FE, SU and AH designed the experiments; FE conducted most experiments and analysed the data; MF carried out and analysed the preference assays over time course; MR provided the method for mannitol and B vitamin analysis; FE wrote the manuscript; all authors reviewed and edited the manuscript.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.931zcrjgq.

REFERENCES

Al-Naemi, F. & Hatcher, P.E. (2013). Contrasting effects of necrotrophic and biotrophic plant pathogens on the aphid Aphis fabae. *Entomol. Exp. Appl.*, 148, 234–245.

Baldrian, P. (2017). Forest microbiome: diversity, complexity and dynamics. *FEMS Microbiol. Rev.*, 41, 109–130.

Barbe, G.D. (1964). Relation of European earwig to snapdragon rust. *Phytopathology*, 54, 369–371.

Barbehenn, R.V., Niewiadomski, J., Kochmanski, J. & Constabel, C.P. (2012). Limited effect of reactive oxygen species on the composition of susceptible esentail amino acids in the midguts of Lymantria dispar caterpillars. *Arch. Insect Biochem. Physiol.*, 81, 160–177.

Benrey, B. & Denno, R.F. (1997). The slow-growth–high-mortality hypothesis: a test using the cabbage butterfly. *Ecology*, 78, 987–999.

Berger, S., Sinha, A.K. & Roitsch, T. (2007). Plant physiology meets phytopathology: plant primary metabolism and plant–pathogen interactions. *J. Exp. Bot.*, 58, 4019–4026.

Biere, A. & Tack, A.J. (2013). Evolutionary adaptation in three-way interactions between plants, microbes and arthropods. *Funct. Ecol.*, 27, 646–660.

Boeckler, G.A., Gershenzon, J. & Unsicker, S.B. (2011). Phenolic glycosides of the Salicaceae and their role as anti-herbivore defenses. *Phytochemistry*, 72, 1497–1509.

Boeckler, G.A., Gershenzon, J. & Unsicker, S.B. (2013). Gypsy moth caterpillar feeding has only a marginal impact on phenolic compounds in old-growth black poplar. *J. Chem. Ecol.*, 39, 1301–1312.

Boeckler, G.A., Towns, M., Unsicker, S.B., Mellway, R.D., Yip, L., Hilke, I. *et al.* (2014). Transgenic upregulation of the condensed tannin pathway in poplar leads to a dramatic shift in leaf palatability for two tree-feeding Lepidoptera. *J. Chem. Ecol.*, 40, 150–158.

Boeckler, G.A., Paetz, C., Feibicke, P., Gershenzon, J. & Unsicker, S.B. (2016). Metabolism of poplar salicinoids by the generalist herbivore *Lymantria dispar* (Lepidoptera). *Insect Biochem. Mol. Biol.*, 78, 39–49.

Browne, L.B. (1995). Ontogenic changes in feeding behavior. In *Regulatory Mechanisms in Insect Feeding* (eds Chapman, R.F. & de Boer, G.). Springer, Boston (USA), pp. 307–342.

Busby, P.E., Peay, K.G. & Newcombe, G. (2016). Common foliar fungi of *Populus trichocarpa* modify *Melampsora* rust disease severity. *New Phytol.*, 209, 1681–1692.

Carruthers, R.I., Bergstrom, G.C. & Haynes, P.A. (1986). Accelerated development of the european corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), induced by interactions with *Colletotrichum graminicola* (Melanconiales: Melanconiaceae), the causal fungus of maize anthracnose. *Ann. Entomol. Soc. Am.*, 79, 385–389.

Crocoll, C., Mirza, N., Reichelt, M., Gershenzon, J. & Halkier, B.A. (2016). Optimization of engineered production of the glucoraphanin precursor dihomomethionine in *Nicotiana benthamiana*. *Frontiers in Bioengineering and. Biotechnology*, 4.

Desurmont, G.A., Xu, H. & Turlings, T.C. (2016). Powdery mildew suppresses herbivore-induced plant volatiles and interferes with parasitoid attraction in *Brassica rapa*. *Plant, Cell Environ.*, 39, 1920–1927.

Douglas, A.E. (2015). Multiorganismal insects: diversity and function of resident microorganisms. *Annual Rreview of Entomology*, 60, 17–34.

Eberl, F., Hammerbacher, A., Gershenzon, J. & Unsicker, S.B. (2018). Leaf rust infection reduces herbivore-induced volatile emission in black poplar and attracts a generalist herbivore. *New Phytol.*, 220, 760–772.

Eberl, F., Uhe, C. & Unsicker, S.B. (2019). Friend or foe? The role of leaf-inhabiting fungal pathogens and endophytes in tree-insect interactions. *Fungal Ecol.*, 38, 104–112.

Elkinton, J. & Liebhold, A. (1990). Population dynamics of gypsy moth in North America. *Annu. Rev. Entomol.*, 35, 571–596.

Fernandez-Conradi, P., Jactel, H., Robin, C., Tack, A.J. & Castagneyrol, B. (2018). Fungi reduce preference and performance of insect herbivores on challenged plants. *Ecology*, 99, 300–311.

Friend, W. (1958). Nutritional requirements of phytophagous insects. *Annu. Rev. Entomol.*, 3, 57–74.

Futuyma, D.J. & Agrawal, A.A. (2009). Macroevolution and the biological diversity of plants and herbivores. *Proceedings of the National Academy of Sciences*: pnas.0904106106.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.*, 43, 205–227.

Hacquard, S., Petre, B., Frey, P., Hecker, A., Rouhier, N. & Duplessis, S. (2011) The poplar-poplar rust interaction: insights from genomics and transcriptomics. *Journal of Pathogens* 2011, 1–11. Article-ID 716041, 716011 pages.

Hammerbacher, A., Schmidt, A., Wadke, N., Wright, L.P., Schneider, B., Bohlmann, J. *et al.* (2013). A common fungal associate of the spruce bark beetle metabolizes the stilbene defenses of Norway spruce. *Plant Physiol.*, 162, 1324–1336.

Hatcher, P.E. (1995). Three-way interactions between plant pathogenic fungi, herbivorous insects and their host plants. *Biol. Rev.*, 70, 639–694.

Jennings, D.B., Ehrenshaft, M., Pharr, D.M. & Williamson, J.D. (1998). Roles for mannitol and mannitol dehydrogenase in active oxygen-mediated plant defense. *Proc. Natl Acad. Sci.*, 95, 15129–15133.

Johnson, S.N., Douglas, A.E., Woodward, S. & Hartley, S.E. (2003). Microbial impacts on plant-herbivore interactions: the indirect effects of a birch pathogen on a birch aphid. *Oecologia*, 134, 388–396.

Khaksari, M., Mazzoleni, L.R., Ruan, C., Song, P., Hershey, N.D., Kennedy, R.T. *et al.* (2018). Detection and quantification of vitamins in microliter volumes of biological samples by LC-MS for clinical screening. *AIChE J.*, 64, 3709–3718.

Lämke, J.S. & Unsicker, S.B. (2018). Phytochemical variation in treetops: causes and consequences for tree-insect herbivore interactions. *Oecologia*, 187, 377–388.

Lindroth, R.L., Klein, K.A., Hemming, J.D. & Feuker, A.M. (1997). Variation in temperature and dietary nitrogen affect performance of the gypsy moth (*Lymantria dispar* L.). *Physiol. Entomol.*, 22, 55–64.

Madsen, S.R., Kunert, G., Reichelt, M., Gershenzon, J. & Halkier, B.A. (2015). Feeding on leaves of the glucosinolate transporter mutant *gtr1gtr2* reduces fitness of *Myzus persicae*. *J. Chem. Ecol.*, 41, 975–984.

Martin, M.M. (1979). Biochemical implications of insect mycophagy. *Biol. Rev.*, 54, 1–21.

Martin, M. & Kukor, J. (1984) Role of mycophagy and bacteriophagy in invertebrate nutrition. Current Perspectives in Microbial Ecology 257.

Mason, C.J., Couture, J.J. & Raffa, K.F. (2014). Plant-associated bacteria degrade defense chemicals and reduce their adverse effects on an insect defoliator. *Oecologia*, 175, 901–910.

Mondy, N., Charrier, B., Fermaud, M., Pracros, P. & Corio-Costet, M.-F. (1998). Olfactory and gustatory behaviour by larvae of *Lobesia botrana* in response to *Botrytis cinerea*. *Entomol. Exp. Appl.*, 88, 1–70.

Moskowitz, D. & Haramaty, L. (2012). Fungi feeding by the agreeable tiger moth (*Spilosoma congrua* Walker) (Erebidae: Arctiinae). *Journal of the Lepidopterists' Society*, 66, 230–232.

Oliveira, C.M., Ferreira, J.A.M., Oliveira, R.M., Santos, F.O. & Pallini, A. (2014). Ricoseius loxocheles, a phytoseiid mite that feeds on coffee leaf rust. *Exp Appl Acarol*, 64, 223–233.

Pei, M. & Shang, Y. (2005). A brief summary of *Melampsora* species on *Populus*. In *Rust Diseases of Willow and Poplar* (eds Pei, M.H. & McCracken, A.R.). CABI, Wallingford, pp. 51–61.

Ramsell, J. & Paul, N.D. (1990). Preferential grazing by molluscs of plants infected by rust fungi. *Oikos*, 58, 145–150.

Rawlings, J.E. (1984). Mycophagy in Lepidoptera. *Fungus-Insect Relationships*. Columbia University Press, New York, pp. 382–423.

Reddy, M. & Rao, A. (1976). Changes in the composition of free and protein amino acids in groundnut leaves induced by infection with *Puccinia arachidis* Speg. *Acta Phytopathologica*, 11, 167–172.

Reding, M.E., Alston, D.G., Thomson, S.V. & Stark, A.V. (2001). Association of powdery mildew and spider mite populations in apple and cherry orchards. *Agr. Ecosyst. Environ.*, 84, 177–186.

Rizvi, S.Z.M., Raman, A., Wheatley, W., Cook, G. & Nicol, H. (2015). Influence of *Botrytis cinerea* (Helotiales: Sclerotiniaceae) infected leaves of *Vitis vinifera* (Vitales: Vitaceae) on the preference of *Epiphyas postvittana* (Lepidoptera: Tortricidae). *Austral Entomology*, 54, 60–70.

Robinson, G.S., Ackery, P.R., Kitching, I.J. & Beccalone, G.W. (2010) Hernández LM HOSTS database. Natural History Museum. http://www.nhm.ac.uk/our-science/data/hostplants/.

Schiff, N.M., Waldbauer, G.P. & Friedman, S. (1989). Response of last instar *Heliothis zea* larvae to carbohydrates: stimulation of biting, nutritional value. *Entomol. Exp. Appl.*, 52, 29–37.

Scriber, J. & Slansky, F. Jr. (1981). The nutritional ecology of immature insects. *Annu. Rev. Entomol.*, 26, 183–211.

Shikano, I., Rosa, C., Tan, C.-W. & Felton, G.W. (2017). Tritrophic interactions: microbe-mediated plant effects on insect herbivores. *Annu. Rev. Phytopathol.*, 55, 313–331.

Stockhoff, B.A. (1993). Protein intake by gypsy moth larvae on homogeneous and heterogeneous diets. *Physiol. Entomol.*, 18, 409–419.

Sutherland, A.M. & Parrella, M.P. (2009). Mycophagy in Coccinellidae: review and synthesis. *Biological Control*, 51, 284–293.

Takada, T., Sato, R. & Kikuta, S. (2017). A mannitol/sorbitol receptor stimulates dietary intake in Tribolium castaneum. *PLoS ONE*, 12, e0186420.

Ullah, C., Tsai, C.-J., Unsicker, S.B., Xue, L., Reichelt, M., Gershenzon, J. *et al.* (2019). Salicylic acid activates poplar defense against the biotrophic rust fungus *Melampsora larici-populina* via increased biosynthesis of catechin and proanthocyanidins. *New Phytol.*, 221, 960–975.

Unsicker, S.B., Oswald, A., Köhler, G. & Weisser, W.W. (2008). Complementarity effects through dietary mixing enhance the performance of a generalist insect herbivore. *Oecologia*, 156, 313–324.

Van Wees, S.C., Van der Ent, S. & Pieterse, C.M. (2008). Plant immune responses triggered by beneficial microbes. *Curr. Opin. Plant Biol.*, 11, 443–448.

Voegele, R.T., Hahn, M., Lohaus, G., Link, T., Heiser, I. & Mendgen, K. (2005). Possible roles for mannitol and mannitol dehydrogenase in the biotrophic plant pathogen *Uromyces fabae*. *Plant Physiol.*, 137, 190–198.

White, T.C. (1993). *The Inadequate Environment: Nitrogen and the Abundance of Animals*. Springer Science & Business Media, Berlin.

Yarwood, C.E. (1943). Associations of thrips with powdery mildews. *Mycologia* 35, 189–191.

Yoshimatsu, S-i & Nakata, Y. (2006). Fungivory of *Anatatha lignea*, an interesting habit in Noctuidae (Lepidoptera). *Entomol. Sci.* 9, 319–325.

Zalucki, M.P., Clarke, A.R. & Malcolm, S.B. (2002). Ecology and behavior of first instar larval Lepidoptera. *Annu. Rev. Entomol.*, 47, 361–393.

Editor, Ted Turlings
Manuscript received 8 December 2019
First decision made 19 January 2020
Second decision made 28 February 2020
Manuscript accepted 8 March 2020