

The role of fungal parasites in tri-trophic interactions involving lichens and lichen-feeding snails

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Received: 7 January 2016

Accepted: 17 March 2016

New Phytologist (2016) 211: 1352–1357

doi: 10.1111/nph.13975

Key words: carbon : nitrogen (C : N) ratio, carbon-based secondary compounds (CBSCs), chemical defence, herbivory, lichenicolous fungi, palatability, parasitism, tri-trophic interaction.

Summary

- Lichens are hosts for a variety of lichenicolous fungi. By investigating two lichens with specialized parasites, we will test the hypothesis that these parasites reduce lichen fitness by increasing the palatability of their respective hosts.
- The palatability of *Lobarina scrobiculata* and *Lobaria pulmonaria* with or without galls of the lichenicolous fungi, *Plectocarpon scrobiculatae* and *P. lichenum*, respectively, were quantified in a feeding-preference experiment with grazing snails (*Cepaea hortensis*). We repeated the experiment for pairs with or without gall in which the carbon-based secondary compounds (CBSCs) had been reduced nondestructively by acetone rinsing.
- Lichens with galls had lower concentration of CBSCs than those without, but this contrast disappeared after acetone rinsing. In the lichen high in nitrogen (N) (the cyanolichen *L. scrobiculata*), the grazing was low, and the snails did not discriminate between specimens with and without *Plectocarpon*-galls. In *L. pulmonaria* low in N (green algae as main photobiont), the parasite reduced the lichen C : N ratio and the snails strongly preferred specimens with *Plectocarpon*-galls, regardless of whether CBSC concentration had been reduced or not.
- In conclusion, some lichen parasites can indirectly reduce lichen fitness by increasing its palatability and thus the grazing pressure from snails, whereas other parasites do not affect grazing preferences.

Introduction

Primary producers such as plants and lichens form the nutritional basis for herbivores as well as parasites and pathogenic microorganisms. Plant-mediated interactions between pathogenic microorganisms and invertebrate herbivores are increasingly studied because of their potential to drive population and community dynamics (Stout *et al.*, 2006). Most studies show negative direct and indirect effects of pathogens on herbivorous invertebrates, whereas some show positive effects (reviewed by Hatcher, 1995; Stout *et al.*, 2006). Pathogen-induced negative effects on herbivorous invertebrates are driven mainly by reduced nutritional quality or by pathogen-induced defence compounds (reviewed by Hatcher, 1995; Stout *et al.*, 2006). Although this issue has been widely explored in the plant–invertebrate literature, the impact of parasitic fungi on lichen–invertebrate interactions remains unexplored. Because lichens are the dominant primary producers in c. 8% of terrestrial biomes (Nash, 2008), such interactions should be studied.

About 1500 species of parasitic (lichenicolous) fungi are known to inhabit lichens and the number is increasing. They differ in host-specialization and virulence (Lawrey & Diederich, 2003). For instance, gall-forming lichenicolous fungi are usually highly species-specific and weakly virulent

parasites (Lawrey & Diederich, 2003), consistent with commensalistic relationships (Hawksworth, 1982). Invertebrates, such as gastropods, lepidopteran larvae, mites and springtails, feed on lichens and may cause severe damage (Benesperri & Tretiach, 2004; Vatne *et al.*, 2010; Černajová & Svoboda, 2014). For instance, lichenivorous snails can shape the distribution of species and influence lichen-dominated vegetation (Gauslaa, 2008; Asplund *et al.*, 2010b; Fröberg *et al.*, 2011). The nutritional quality of the food items drives the feeding preference of snails; leaf carbon : nitrogen (C : N) is one important driver of snail feeding preference (Pérez-Harguindeguy *et al.*, 2003). The abundance of mites and springtails positively correlates with lichen thallus N (Bokhorst *et al.*, 2015). Meanwhile, snails' relationship to lichen thallus N content is less clear (Asplund *et al.*, 2010a; Asplund & Wardle, 2013). Many lichens produce carbon-based secondary compounds (CBSCs) that deter lichen-feeding invertebrates (Gauslaa, 2005; Pöykkö *et al.*, 2005; Asplund & Wardle, 2013), as well as lichenicolous fungi (Lawrey, 1989, 2000). A previous study shows that lichens infested with a gall-forming lichenicolous fungus have lower concentrations of CBSCs (Merinero *et al.*, 2015). Yet, we do not know whether lichenicolous fungi affect the herbivore defence of their host, or if they just infect the lichen specimens low in CBSCs. Thus, it is of great interest to

understand the role of lichenicolous fungi in the interaction between lichens and invertebrate herbivores.

We focus on the widespread host–parasite system formed by the epiphytic lichens *Lobaria pulmonaria* and *Lobaria scrobiculata*, and their lichenicolous fungi *Plectocarpon lichenum* and *P. scrobiculatae*, respectively (Ertz *et al.*, 2005). These two lichens also serve as fodder for generalist gastropods such as *Cepaea hortensis* inhabiting forests where it feeds on epiphytic lichens (Asplund *et al.*, 2010b). This tri-trophic interaction likely provides a good model to evaluate the joint effects of parasitism and grazing on lichen fitness. Here, we aim to investigate how the presence of galls of these specialized lichenicolous fungi affects the palatability of their respective lichen host, by testing two hypotheses. First, thalli of *L. scrobiculata* and *L. pulmonaria* hosting galls of *P. scrobiculatae* and *P. lichenum*, respectively, will be grazed more than thalli without galls by the generalist snail *Cepaea hortensis*. This hypothesis was motivated by the consistently lower amount of defence compound concentrations reported in *L. scrobiculata* hosting *P. scrobiculatae* than in those without (Merinero *et al.*, 2015). Second, reducing the concentrations of CBSCs by means of the nondestructive acetone rinsing method (Solhaug & Gauslaa, 2001) will increase the grazing of the thalli, but reduce the grazing preference for thalli with galls. We will further explore whether changes in palatability due to fungal infection can be attributed to changes in nutritional quality of the host. By testing these hypotheses, we aim to advance the understanding of the complexities of defence mechanisms and how lichenicolous fungi may determine the outcome of tri-trophic interactions.

Materials and Methods

Study species

In this study, we used the two N-fixing lichens *Lobaria scrobiculata* (Scop.) Nyl. ex Croum and *Lobaria pulmonaria* (L.) Hoffm. The cyanolichen *L. scrobiculata* contains *Nostoc* as its only photobiont, whereas *L. pulmonaria* has green algae as its main photobiont and *Nostoc* localized in scattered internal cephalodia. The lichenicolous fungus *Plectocarpon scrobiculatae* Diederich & Etayo exclusively inhabits *L. scrobiculata*, whereas its close relative, *Plectocarpon lichenum* (Sommerf.) D. Hawksw., grows on *L. pulmonaria* and two *Pseudocyphellaria* species (Ertz *et al.*, 2005). *Plectocarpon* induces the formation of basally constricted galls on the lichen surface. Ascومات (fruiting bodies) develop on the upper surface of the gall that reproduces sexually and asexually by ascospores and conidia, respectively (Ertz *et al.*, 2005). Both lichen species were collected in two oak (*Quercus pyrenaica* Willd.) forests in central Spain. *Lobaria scrobiculata* with and without *P. scrobiculatae*-galls was collected in Cigüeñelas, Ciudad Real (39°29'27"N, 04°36'33"W; 797 m above sea level (asl); mean annual rainfall 638 mm; mean annual temperature 13.6°C). *Lobaria pulmonaria* with and without *P. lichenum*-galls was collected from a more humid and cooler location in Montejo de la Sierra, Madrid (41°06'44"N, 03°29'33"W; 1263 m asl, 818 mm and 9.5°C of annual rainfall and mean temperature,

respectively) (climate data extracted from CLIMOEST; Sánchez-Palomares *et al.*, 1999). For each thallus with galls, we collected a similar-sized specimen without galls from the same tree. Sampling was done on 14 March 2015 and 30 June 2015 for *L. scrobiculata* and *L. pulmonaria*, respectively. For each lichen species, we randomly collected 20 thalli without galls and 20 thalli with *Plectocarpon*-galls from various random trees.

In order to evaluate the palatability of the lichens, we used the snail *Cepaea hortensis* (Helicidae) that is widespread in Europe. This is a generalist grazer measuring 14–20 mm wide that feeds naturally on lichens (including the study species) as well as on forbs (Speiser, 2001; Asplund & Gauslaa, 2008; Asplund *et al.*, 2010b). The snails were fed with the respective lichen species for 1 d and then starved for 24 h before the start of the experiments.

Experimental design

All air-dry thalli were dried further in a desiccator for 24 h and then 10 of each category were submerged in 100% acetone (c. 50 ml per 100 mg lichen) for 40 min. The containers with acetone were closed with Parafilm during the extraction to avoid evaporation. Afterwards, all thalli were gently blotted with a paper towel and left for some hours until all acetone had evaporated. Each *L. pulmonaria* thallus was cut in two parts: one part was for chemical analyses, the other for the feeding experiment. The *L. scrobiculata* thalli were too small to allow both the feeding experiment and the chemical analyses. For each lichen species and each acetone treatment, 10 plastic boxes (in total 40 boxes; 10 × 7 × 6 cm) were set up. One thallus with and one without galls (preweighed ± 0.1 mg) were placed in each box; the lichens were sprayed with 3 ml water and three randomly selected snails were placed in each box. The boxes were then closed with a perforated lid and left for 48 h at room temperature and in natural daylight shaded from direct sunlight (as described by Gauslaa, 2005). Afterwards, each thallus was air dried and subsequently reweighed.

Concentrations of C, N and CBSCs were analysed in both acetone-rinsed and control thalli of *L. pulmonaria*. First, the *P. lichenum*-galls were removed from the thalli with a tweezer and analysed separately. Then we ground each of the three categories – thalli without galls; thalli hosting galls but with galls removed; and *P. lichenum* galls – separately to a fine powder in a ball mill (MM400; Retsch GmbH, Haan, Germany). Approximately 30 mg of the powder was extracted in 2 ml acetone three times for 50 min. The combined supernatants were evaporated and re-dissolved in 500–1000 µl acetone. The extracted compounds were then quantified by HPLC using an ODS Hypersil column, 50 × 4.6 mm using 0.25% orthophosphoric acid and 1.5% tetrahydrofuran in Millipore water (A) and 100% methanol (B) as mobile phases at 2 ml min⁻¹, and UV detection at 245 nm, following Nybakken *et al.* (2007). Compound identification was based on retention times, online UV spectra and co-chromatography of commercial standards. Carbon and N was analysed with an Elementar Vario MICRO cube (Elementar Analysensysteme GmbH, Hanu, Germany). Element analyses of *L. scrobiculata* were done on additional thalli and not those used in the feeding experiment. Unfortunately, this *L. scrobiculata*

material was insufficient for analysis of CBSCs. However, Merinero *et al.* (2015) showed that the concentration of both medullary and cortical compounds were about twice as high in *L. scrobiculata* without galls than in those hosting *P. scrobiculatae* galls.

Statistical analysis

Preference, *sensu* Lockwood (1998), was calculated as the biomass consumed of one thallus divided by the pooled consumption of both thalli in the box (hereafter referred to as feeding preference, expressed as a percentage). Thus, when the snails consumed equal amounts of each thallus, the preference was 50% for both thalli. We used Student's *t*-tests to test for the effect of presence vs absence of *Plectocarpon*-galls on feeding preference. In order to test for difference in nutrients and CBSCs in *L. pulmonaria* between the two treatments, we used a paired *t*-test. For *L. scrobiculata*, we performed an ordinary *t*-test instead because these data were from material not used in the experiment and thus not paired. For each box with *L. pulmonaria*, we calculated the ratio between the concentration of CBSCs in the thallus with galls and the concentration of CBSCs in the thallus without galls ($\text{CBSC}_{\text{with galls}}/\text{CBSC}_{\text{without}}$). This was also done with the nutrient measurements yielding the parameters $[\text{N}]_{\text{with galls}}/[\text{N}]_{\text{without}}$, $[\text{C}]_{\text{with galls}}/[\text{C}]_{\text{without}}$ and $[\text{C}:\text{N}]_{\text{with galls}}/[\text{C}:\text{N}]_{\text{without}}$. Clearly, when these ratios are >1 , the thallus with galls has a higher concentration of nutrients and CBSCs than the thallus without galls. We then performed Kendall's tau correlation tests between these ratios and the preference thallus with galls. A positive correlation indicated that the preference is stronger when the difference in nutrients or CBSCs between the two thalli is larger. All statistical tests were performed in R (v.3.2.0; R Development Core Team, Vienna, Austria).

Results

Lobaria pulmonaria with and without galls contained stictic acid, constictic acid, norstictic acid, methyl norstictic acid and peristictic acid ranked in order of (falling) concentration (Table 1). However, one specimen without galls contained norstictic acid only. The total CBSC concentration was 1.64 times higher in thalli without galls than in those with galls. Acetone rinsing reduced the total CBSC concentration to 55% and 43% of original concentration in thalli with and without galls, respectively. Due to the lower extraction efficiency for CBSCs in thalli with galls, there was no significant difference in total CBSC concentration between thalli with and without galls after acetone rinsing (Table 1). Yet, peristictic acid, representing only 0.6% of the total CBSC pool, was significantly higher in thalli without galls (Table 1). The removed galls contained approximately the same concentrations of CBSC as the thalli from which they had been cut (Table 2; total CBSC (mg g^{-1}) thallus: 25.1 ± 3.0 , galls: 33.3 ± 9.0 ; $t = 1.14$, $P = 0.297$, paired *t*-test). However, concentration of the minor CBSC, methyl norstictic acid, was 3.8 times higher in the thallus than in the galls (Table 2; $t = 6.43$, $P < 0.001$, paired *t*-test). Acetone rinsing significantly reduced

Table 1 Carbon-based secondary compounds (CBSCs, mg g^{-1} ; mean ± 1 SE) in *Lobaria pulmonaria* without *Plectocarpon*-galls as well as thalli hosting galls of the lichenicolous fungi *P. lichenum*

	Without galls	With galls	<i>t</i> (<i>P</i>)
<i>Controls</i>			
Stictic acid	27.86 ± 4.8	19.17 ± 1.5	1.85 (0.097)
Constictic acid	8.39 ± 1.6	5.52 ± 0.9	1.96 (0.082)
Norstictic acid	7.02 ± 2.8	1.67 ± 0.3	1.81 (0.104)
Methyl norstictic acid	0.39 ± 0.1	0.26 ± 0.03	2.09 (0.067)
Peristictic acid	0.21 ± 0.03	0.12 ± 0.01	2.85 (0.019)
Total CBSCs	43.88 ± 5.7	26.73 ± 2.5	2.79 (0.021)
<i>Acetone-rinsed</i>			
Stictic acid	13.66 ± 1.6	10.91 ± 1.6	1.25 (0.242)
Constictic acid	3.63 ± 0.6	3.07 ± 0.8	0.72 (0.491)
Norstictic acid	0.98 ± 0.2	0.59 ± 0.1	1.62 (0.139)
Methyl norstictic acid	0.13 ± 0.01	0.12 ± 0.02	0.67 (0.519)
Peristictic acid	0.17 ± 0.01	0.08 ± 0.01	5.44 (<0.001)
Total CBSCs	18.65 ± 2.1	14.77 ± 2.5	1.25 (0.243)

Control thalli have natural levels of CBSCs but acetone rinsing reduced the concentration. Values in bold are significantly different (at $P < 0.05$, $\text{df} = 9$) according to a paired *t*-test.

Table 2 Carbon-based secondary compounds (CBSCs, mg g^{-1} ; mean ± 1 SE), nitrogen (N, %), carbon (C, %) and C : N in galls and thallus of *Lobaria pulmonaria* infected with the lichenicolous fungus *Plectocarpon lichenum*

	Thallus	Galls	<i>t</i> (<i>P</i>)
<i>Controls</i>			
Stictic acid	18.91 ± 2.1	19.39 ± 4.2	0.17 (0.873)
Constictic acid	4.50 ± 0.8	10.84 ± 4.0	1.70 (0.139)
Norstictic acid	1.34 ± 0.2	2.76 ± 0.9	1.80 (0.122)
Methyl norstictic acid	0.23 ± 0.04	0.06 ± 0.02	6.43 (<0.001)
Peristictic acid	0.10 ± 0.01	0.22 ± 0.1	1.44 (0.199)
Total CBSCs	25.08 ± 3.0	33.27 ± 9.0	1.14 (0.297)
Nitrogen	2.28 ± 0.1	2.30 ± 0.1	0.38 (0.728)
Carbon	41.20 ± 0.3	42.82 ± 0.2	4.52 (0.020)
C : N	18.17 ± 0.5	18.67 ± 0.4	1.12 (0.345)
<i>Acetone-rinsed</i>			
Stictic acid	10.18 ± 2.3	16.30 ± 2.5	6.15 (<0.001)
Constictic acid	2.63 ± 1.0	7.11 ± 1.5	5.56 (0.001)
Norstictic acid	0.59 ± 0.1	1.59 ± 2.5	3.35 (0.015)
Methyl norstictic acid	0.11 ± 0.02	0.06 ± 0.03	4.70 (0.003)
Peristictic acid	0.08 ± 0.02	0.21 ± 0.04	2.66 (0.038)
Total CBSCs	13.59 ± 3.5	25.26 ± 4.2	6.56 (<0.001)
Nitrogen	2.26 ± 0.1	2.14 ± 0.1	5.94 (0.004)
Carbon	41.34 ± 0.3	43.43 ± 0.2	7.03 (0.002)
C : N	18.35 ± 0.4	20.39 ± 0.7	6.73 (0.003)

Control thalli have natural levels of CBSCs but acetone rinsing reduced the concentration. Values in bold are significantly different (at $P < 0.05$) according to a paired *t*-test. $\text{df} = 6$ (CBSC data), $\text{df} = 4$ (element data of acetone rinsed thalli), $\text{df} = 3$ (element data of control thalli).

total CBSCs in the host thalli from 25.1 ± 3.0 to $13.6 \pm 3.5 \text{ mg g}^{-1}$ (Table 2; $t = 2.51$, $P = 0.028$, *t*-test), but not in the removed galls as such ($t = 0.81$, $P = 0.441$, *t*-test).

Carbon concentration in *L. pulmonaria* was significantly higher in thalli without galls than in those hosting *Plectocarpon* (Table 3). However, after reducing CBSCs concentration by acetone rinsing, C no longer differed between thalli with and

Table 3 Percentage of carbon (C), nitrogen (N) and C : N (mean \pm 1 SE) in *Lobaria pulmonaria* and *Lobaria scrobiculata* without *Plectocarpon*-galls as well as thalli hosting galls of the lichenicolous fungi *P. lichenum* and *P. scrobiculatae*, respectively

	Without galls	With galls	<i>t</i> (<i>P</i>)
<i>L. pulmonaria</i> , Controls			
Carbon	43.03 \pm 0.3	41.65 \pm 0.3	3.15 (0.012)
Nitrogen	1.97 \pm 0.1	2.16 \pm 0.05	2.23 (0.053)
C : N	22.11 \pm 0.9	19.36 \pm 0.6	2.85 (0.019)
<i>L. pulmonaria</i> , Acetone-rinsed			
Carbon	41.93 \pm 0.4	42.22 \pm 0.6	0.51 (0.625)
Nitrogen	1.97 \pm 0.1	2.18 \pm 0.1	2.61 (0.028)
C : N	21.53 \pm 0.8	19.53 \pm 0.8	2.77 (0.022)
<i>L. scrobiculata</i> , Controls			
Carbon	43.61 \pm 0.4	44.06 \pm 0.4	0.84 (0.411)
Nitrogen	2.77 \pm 0.05	2.84 \pm 0.08	0.80 (0.434)
C : N	15.86 \pm 0.4	15.66 \pm 0.5	0.32 (0.752)

Control thalli have natural levels of carbon based secondary compounds (CBSCs) but acetone rinsing reduced the concentration. Values in bold are significantly different (at $P < 0.05$, $df = 9$) according to a paired *t*-test (*L. pulmonaria*). Samples of *L. scrobiculata* were not paired and therefore an ordinary *t*-test ($df = 24$) was performed.

without galls. *Lobaria pulmonaria* with galls had lower C : N than thalli without galls for both controls and acetone-rinsed thalli, whereas N was only significantly higher in the thalli with galls after rinsing (Table 3). For *L. scrobiculata*, that has substantially higher N concentration than *L. pulmonaria*, C, N and C : N did not vary between thalli with and without galls (Table 3).

The snails showed no preference for *L. scrobiculata* with *Plectocarpon*-galls or thalli without galls (Fig. 1a), regardless of whether CBSCs were at natural levels ($t = 0.068$, $df = 8$, $P = 0.947$, *t*-test) or had been reduced in concentration ($t = 0.196$, $df = 8$, $P = 0.846$, *t*-test). By contrast, for *L. pulmonaria*, snails preferred thalli with galls to those without galls (Fig. 1b; controls $W = 5$, $P < 0.001$; acetone-rinsed: $W = 6$, $P < 0.001$, Wilcoxon rank-sum test). Reducing the CBSC concentration (to equally low levels for both treatments) increased the total amount of biomass grazed (see inserted mean values in Fig. 1), but did not increase the preference in any of the lichen hosts. In both species, the snails avoided feeding on the *Plectocarpon*-galls (Fig. 2).

There was a negative correlation between the preference for *L. pulmonaria* with galls and $C:N_{\text{with galls}}/C:N_{\text{without}}$ (Fig. 3; $r = -0.532$, $n = 20$, $P = 0.016$, Pearson). This means that snails tended to prefer a thallus with galls more when it had a lower C : N ratio than its paired thallus without galls. There was no significant correlation between preference for the thallus galls and either $[CBSC]_{\text{with galls}}/[CBSC]_{\text{without}}$ ($r = 0.150$, $n = 18$, $P = 0.552$, Pearson), $[N]_{\text{with galls}}/[N]_{\text{without}}$ ($r = 0.387$, $n = 20$, $P = 0.092$, Pearson) or $[C]_{\text{with galls}}/[C]_{\text{without}}$ ($r = -0.284$, $n = 20$, $P = 0.086$, Kendall's tau).

Discussion

This study documents for the first time the joint effects of parasitism and grazing (multi-trophic interaction) on the fitness of lichen hosts with natural and reduced concentrations of

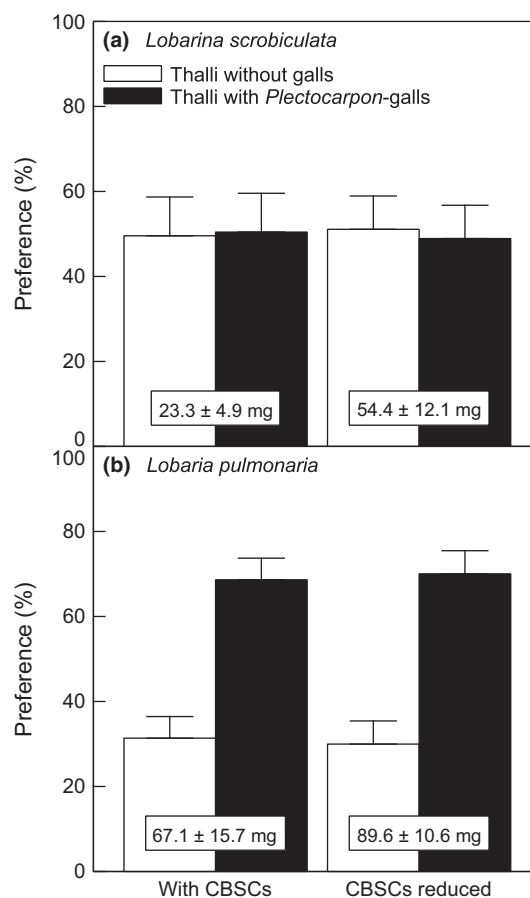


Fig. 1 Feeding preference (expressed as percentage of total consumption, mean \pm SE; $n = 10$) of the snail *Cepaea hortensis* when given the choice between thalli with and without galls of the lichenicolous fungi *Plectocarpon* sp. Snails were offered thalli with natural levels of carbon-based secondary compounds (CBSCs) and with CBSCs levels reduced by means of acetone rinsing. (a) *Lobaria scrobiculata* infected with *P. scrobiculatae* and (b) *Lobaria pulmonaria* infected with *P. lichenum*. Values in boxes represent total consumption (mean \pm SE; $n = 10$).

carbon-based secondary compounds (CBSCs). The generalist snail *Cepaea hortensis* preferred feeding on *L. pulmonaria* hosting galls of the lichenicolous fungus *P. lichenum*, despite the fact that it avoided the parasite galls as such. By contrast, the lichenicolous fungus *Plectocarpon scrobiculatae* did not affect the palatability of its host, *L. scrobiculata*, meaning that our first hypothesis was supported for only one of two studied lichens. One possible explanation for the snails' preference for *L. pulmonaria* with galls is their lower carbon-to-nitrogen ratio (C : N), which is consistent with an increasing preference for the thalli with galls with an increasing difference in C : N between thalli with galls vs thalli without galls (Fig. 3). For vascular plants, C : N is a good estimate of nutritional quality; snails prefer feeding on leaves with low C : N (Pérez-Harguindeguy *et al.*, 2003). Our findings that parasitic fungi increased the palatability of *L. pulmonaria* contrasts with many studies of tri-trophic interactions involving plants (Hatcher, 1995; Stout *et al.*, 2006). However, pathogens may increase the palatability of some plants; peanut plants infested with white mold, *Sclerotium rolfsii*, are preferred over healthy plants by the beet armyworm (Cardoza *et al.*, 2002, 2003). For



Fig. 2 *Lobaria pulmonaria* grazed by *Cepaea hortensis*. Note that the black *Plectocarpon lichenum*-galls are avoided. Bar, 5 mm.

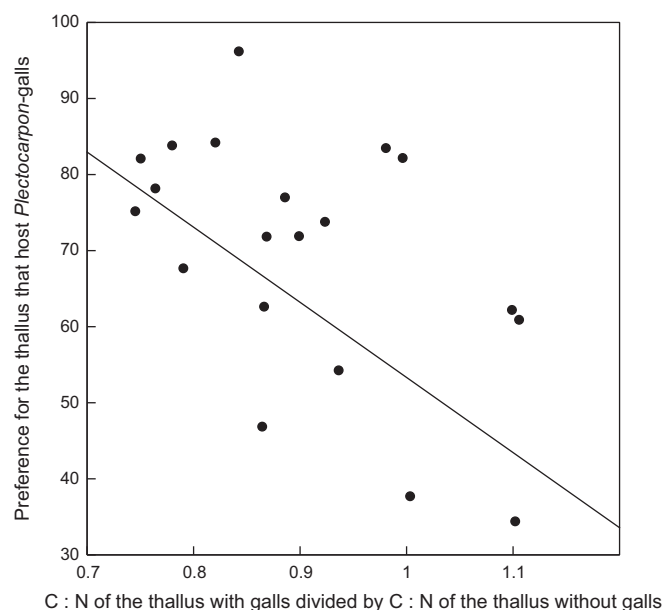


Fig. 3 Relationship between the preference of *Cepaea hortensis* for *Lobaria pulmonaria* thalli with galls and carbon: nitrogen (C : N)_{with galls}/C : N_{without galls}.

the herb *Cirsium arvense*, inoculation with various endophytic fungi lowered its C : N ratio and the inoculated leaves were preferred by a specialist insect (Gange *et al.*, 2011). When there is a pathogenic-induced reduction in invertebrate grazing on plants, this is usually ascribed to lowered nutritional quality or induced defence (Hatcher *et al.*, 2006).

The presence of galls did not affect the major nutrients C and N of *L. scrobiculata*, which is higher in N than *L. pulmonaria*. This may explain the lack of response in feeding preference on *L. scrobiculata*. In line with our findings, earlier studies showed that microbial infection affected palatability differently in the two host species. Partly senesced *L. pulmonaria* thalli placed

below forest litter and thus exposed to various decomposing microbes were more palatable to *C. hortensis* than healthy thalli transplanted to tree trunks, whereas *L. scrobiculata* became less palatable after senescence had started (Asplund & Wardle, 2012).

We found no support for the second hypothesis predicting that the grazing preference for thalli with *Plectocarpon*-galls is due to their lower CBSC concentrations. Indeed, *L. pulmonaria* with *P. lichenum*-galls did contain less CBSCs than thalli without galls. Nevertheless, the snails' preference for thalli with galls was just as high after the acetone rinsing, which eliminated the contrast in CBSC concentration between thalli with and without galls. Furthermore, increasing differences in CBSC concentration between thalli with and without galls did not affect the preference for the thallus with galls, as shown by the nonsignificant correlation between [CBSC]_{with galls}/[CBSC]_{without} and preference for the thallus with galls. In fact, in the boxes where CBSC concentration was similar between the treatments, the snails still preferred the thallus with galls. These results are surprising considering that the palatability of *L. pulmonaria* increases considerably following acetone rinsing (Gauslaa, 2005; Asplund & Gauslaa, 2008).

The fact that galls contain at least as much CBSCs as the normal host thallus itself, suggests that galls consist mainly of the mycobiont in the lichen host. Thereby, *Plectocarpon* probably contributes little to total gall biomass. This is not necessarily a general response of lichenicolous fungi; for example, Grube & de los Ríos (2001) did not find CBSCs in *Biatoropsis usnearum*-galls on *Usnea rigida*. The lack of significant CBSC concentration gradient between lichen thallus and galls suggests that there is hardly any localized parasite-induced depression of secondary metabolism in the lichen specimen. Thus, the lower CBSC concentration measured for entire lichen thalli with *Plectocarpon*-galls are caused either by an avoidance strategy by selection of thalli low in CBSC, or there is some kind of parasite-induced downregulation of lichen secondary metabolism (Panstruga, 2003; Schulze-Lefert & Panstruga, 2003).

Conclusions

Lichen parasites, even within the same genus, affect tri-trophic relationships (lichen–parasite–herbivore) differently. In the lichen species highest in N, the grazing was low, and the snails did not discriminate between specimens with and without *Plectocarpon*-galls. In the lichen low in N, the parasite apparently reduced the C : N ratio and the snails strongly preferred specimens with *Plectocarpon*-galls. For this lichen species, the parasite indirectly reduces lichen fitness by increasing its palatability and thus the grazing pressure from snails. Even though herbivore-deterrent CBSCs had consistently lower concentrations in both lichen species with galls, these CBSCs did not influence the preference, as evidenced by similar preferences for acetone-rinsed pairs in which there were no significant difference in carbon-based defence compounds.

Acknowledgements

We thank Knut Asbjørn Solhaug for collecting *C. hortensis*.

Author contributions

J.A., Y.G. and S.M. planned and designed the research. S.M. conducted field work and J.A. performed experiments and analysed data. J.A., Y.G. and S.M. wrote the manuscript.

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