



**HAL**  
open science

## Overcoming deterrent metabolites by gaining essential nutrients A lichen/snail case study

Alice Gadea, Maryvonne Charrier, Mathieu Fanuel, Philippe Clerc, Corentin Daugan, Aurélie Sauvager, Hélène Rogniaux, Joël Boustie, Anne-Cécile Le Lamer, Françoise Lohezic-Le Devehat

### ► To cite this version:

Alice Gadea, Maryvonne Charrier, Mathieu Fanuel, Philippe Clerc, Corentin Daugan, et al.. Overcoming deterrent metabolites by gaining essential nutrients A lichen/snail case study. *Phytochemistry*, 2019, 164, pp.86-93. 10.1016/j.phytochem.2019.04.019 . hal-02150227

**HAL Id: hal-02150227**

**<https://univ-rennes.hal.science/hal-02150227>**

Submitted on 18 Feb 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Overcoming deterrent metabolites by gaining essential nutrients: a lichen/snail case study**

1 Gadea Alice <sup>a,b</sup>, Charrier Maryvonne <sup>b</sup>, Fanuel Mathieu <sup>c</sup>, Clerc Philippe <sup>d</sup>, Daugan Corentin <sup>a</sup>, Sauvager  
2 Aurélie <sup>a</sup>, Rogniaux Hélène <sup>c</sup>, Boustie Joël <sup>a</sup>, Le Lamer Anne-Cécile <sup>e\*</sup> and Lohézic – Le Devehat Françoise  
3 <sup>a\*¥</sup>

4

5 <sup>a</sup> Univ Rennes, CNRS, ISCR (Institut des Sciences Chimiques de Rennes) - UMR 6226, F-35000 Rennes,  
6 France

7 <sup>b</sup> Univ Rennes, CNRS, ECOBIO (Ecosystèmes, biodiversité, évolution) - UMR 6553, F-35000 Rennes,  
8 France

9 <sup>c</sup> INRA, UR1268 Biopolymers Interactions Assemblies, F-44316 Nantes, France

10 <sup>d</sup> Conservatoire et Jardin Botanique, Département de la culture et du sport, chemin de l'impératrice 1,  
11 1292 Chambésy, Suisse

12 <sup>e</sup> UMR152 PharmaDev, Université de Toulouse, IRD, UPS, F-31400 Toulouse, France

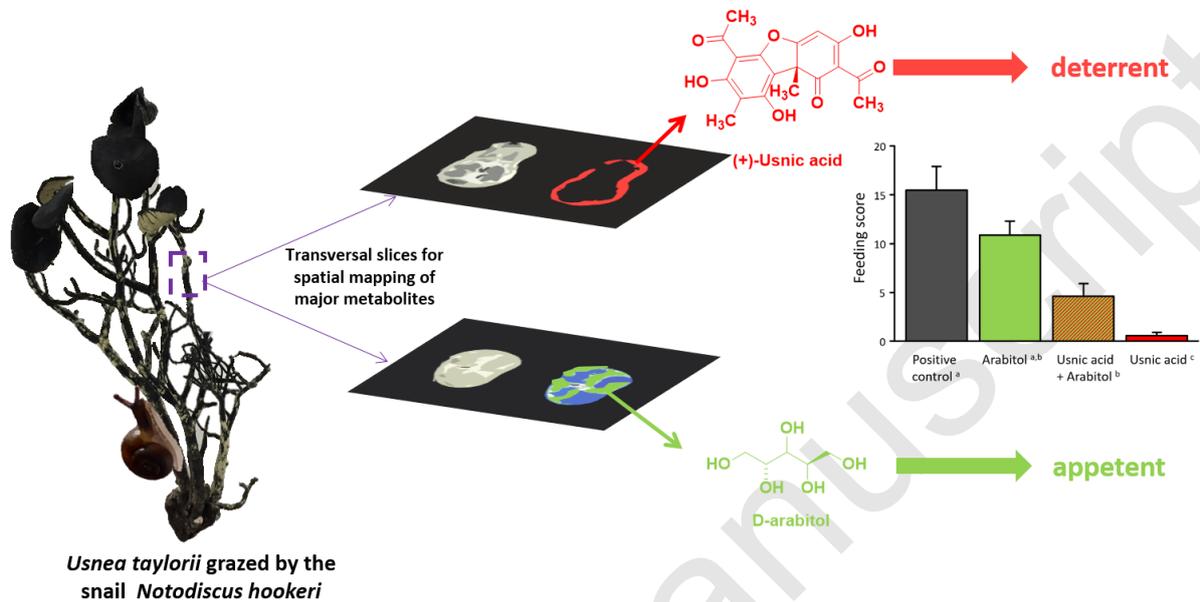
13

14 \* Corresponding author: [françoise.le-devehat@univ-rennes1.fr](mailto:françoise.le-devehat@univ-rennes1.fr) ; + 33 2 23 23 48 16 ; UFR  
15 Pharmacie, Université de Rennes1, 2 avenue du Professeur Léon Bernard, 35043 Rennes cedex, France.

16 ¥ These authors contributed equally to the work

### Graphical abstract

The main primary and specialised metabolites in *Usnea taylorii* are *in situ* located by mass spectrometry imaging. Arabitol, which is highly palatable to the snail *Notodiscus hookeri*, counterbalances the deterrent effect of usnic acid and plays a key role in lichen-snail trophic interactions.



### Highlights

- The lichen *Usnea taylorii* contains mainly usnic acid and arabitol.
- *In situ* mass spectrometry imaging reveals the spatial mapping of the metabolites.
- The land snail *Notodiscus hookeri* consumes lichen parts containing both metabolites.
- The nutritional activity of snails is governed by the chemical quality of the lichen.
- Primary metabolites overcome the deterrent effect of specialised metabolites.

### *Abstract*

Specialised metabolites in lichens are generally considered repellent compounds by consumers. Nevertheless, if the only food available is lichens rich in specialised metabolites, lichenophages must implement strategies to overcome the toxicity of these metabolites. Thus, the balance between phagostimulant nutrients and deterrent metabolites could play a key role in feeding preferences. To further understand lichen-gastropod interactions, we studied the feeding behaviour and consumption in *Notodiscus hookeri*, the land snail native to sub-Antarctic islands. The lichen *Usnea taylorii* was used because of its simple chemistry, its richness in usnic acid (specialised metabolite) and arabitol (primary metabolite) and its presence in snail habitats. Choice tests in arenas with intact lichens *versus* acetone-rinsed lichens were carried out to study the influence of specialised metabolites on snail behaviour and feeding preference. Simultaneously, usnic acid and arabitol were quantified and located within the lichen thallus using HPLC-DAD-MS and *in situ* imaging by mass spectrometry to assess whether their spatial distribution explained preferential snail grazing. No-choice feeding experiments, with the pure metabolites embedded in an artificial diet, defined a gradual gustatory response, from strong repellence (usnic acid) to high appetite (D-arabitol). This case study demonstrates that the nutritional activity of *N. hookeri* is governed by the chemical quality of the food and primarily by nutrient availability (arabitol), despite the presence of deterrent metabolite (usnic acid).

*Keywords:* *Usnea taylorii*, Parmeliaceae, *Notodiscus hookeri*, Mass spectrometry imaging, feeding choice, lichen, snail, usnic acid, D-arabitol

## 17 1. Introduction

18 Lichen symbiosis comprises a fungus, the mycobiont, and photosynthetic partners, the photobiont  
19 (chlorophytes and/or cyanobacteria). The mycobiont produces water-soluble specialised metabolites  
20 such as mycosporines (Roullier et al., 2011) and lectines (Huneck and Yoshimura, 1996) but also  
21 hydrophobic metabolites such as depsides, depsidones and dibenzofurans (Stocker-Wörgötter, 2008).  
22 Hydrophobic metabolites accumulate as tiny crystals outside the fungal hyphae, in the cortex or the  
23 medulla of the lichen thallus (Honegger, 1991). This extracellular localisation makes the metabolites  
24 easy to remove using the acetone-rinsing methodology, without degrading the viability and palatability  
25 of the lichen (Pöykkö et al., 2005; Solhaug and Gauslaa, 2001).

26 Because lichens are sessile and slow-growing symbiotic organisms, they must defend themselves  
27 strongly against lichenivores such as gastropods, which are important lichen feeders (Asplund and  
28 Wardle, 2016; Fröberg et al., 2011; Solhaug and Gauslaa, 2012; Vatne et al., 2010). Many experiments  
29 have highlighted deterrent and/or toxic activity for some specialised metabolites towards  
30 invertebrates, including snails (Emmerich et al., 1993; Gauslaa, 2005; Solhaug and Gauslaa, 2012).  
31 Generalist lichenophage species prefer lichens with few specialised metabolites (Benesperi and  
32 Tretiach, 2004; Boch et al., 2015; Černajová and Svoboda, 2014; Gauslaa, 2005; Goga et al., 2015), but  
33 more than the total amount of metabolites, their nature turns out to be the main discriminant factor  
34 in food preference. In the accidental degradation of the lichen herbarium of the Trieste University, the  
35 coleopteran *Lasioderma serricorne* avoided lichen species containing the dibenzofuran usnic acid, the  
36 depside atranorin or the depsidone fumarprotocetraric acid (Nimis and Skert, 2006). In a similar way,  
37 when snails faced a mixture of *Lobaria pulmonaria* chemotypes with and without stictic acid  
38 (depsidone), a preferential grazing on the depsidone-free lichen was observed (Asplund, 2011a).

39 If the only available resources are lichens rich in specialised metabolites, lichenophages must  
40 overcome or be able to metabolise potentially toxic specialised metabolites (Gadea et al., 2017;  
41 Hesbacher et al., 1995). Thus, optimal foraging in generalist species implies a balance between  
42 defending against toxics and accessing useful nutrients. Indeed, lichen tissues also contain  
43 carbohydrates, including polysaccharides and reserve carbohydrates such as sugars and polyols. The  
44 phagostimulant properties of sugars have previously been highlighted to improve slug baits,  
45 particularly disaccharides that appeared more attractive than monosaccharides (Clark et al., 1997;  
46 Henderson et al., 1992). Gastropods have the full enzymatic arsenal to degrade and to assimilate  
47 sugars and polyols. However, the role of polyols in their feeding choices has not yet been confirmed  
48 (Charrier et al., 2006; Charrier and Rouland, 2001; Flari et al., 1995).

49 As the sub-Antarctic snail *Notodiscus hookeri* Reeve (Charopidae) is an exclusive lichen feeder, it  
50 appears to be a relevant model to advance the understanding of the chemical ecology of the  
51 lichen/snail interactions. In our previous work, a compromise between appetent polyols and

52 potentially repulsive specialised metabolites was suggested. *N. hookeri* is able to deal with potential  
53 toxic metabolites (Gadea et al., 2017) and to select lichen parts according to their gustatory quality  
54 (Gadea et al., 2018). Lichens are known to have a sectorial distribution of their specialised metabolites,  
55 which can be visualised by spectroscopic experiments as LDI-MSI or vibrational spectroscopy (Le  
56 Pogam et al., 2016; Liao et al., 2010). However, it is still not known how the primary metabolites such  
57 as sugars and polyols are distributed within the lichen thallus.

58 In this study, we present the first spatial mapping of both attractive and repellent metabolites to  
59 account for the nutritional choices made by the snail. To address this question, the lichen *Usnea taylorii*  
60 Hook. f. and Taylor (Parmeliaceae) was selected because of its simple chemistry. First, we followed the  
61 snail behaviour in feeding choice arenas to assess the defensive-compound hypothesis described by  
62 Gauslaa (2005). We expected that acetone-rinsed thalli would promote food consumption compared  
63 to control thalli. At the same time, snails were subjected to no-choice feeding experiments using the  
64 pure metabolites embedded in an artificial diet, separately or together. We hypothesised a gradual  
65 gustatory response, from strong repellence (usnic acid) to high appetite (D-arabitol). Second, we  
66 intended to explore the spatial localisation of the main primary (D-arabitol) and specialised (usnic acid)  
67 metabolites of *U. taylorii* using *in-situ* mass spectrometry imaging. The patterns of distribution of the  
68 metabolites were expected to influence the snail feeding strategy.

69

## 70 2. Results

### 71 2.1. Morphological and chemical characterisation of *Usnea taylorii*

#### 72 2.1.1. Description of *Usnea taylorii* morphology

73 Microscopic analysis of the fruticose lichen *Usnea taylorii* reveals a specific morphology that deserves  
74 a detailed description to better understand the location of metabolites (Fig. 1a; a complete  
75 morphological description of the lichen is available in Text S1). Apothecia (the reproductive parts of  
76 the lichen) are located at the apex of the *Usnea* branches and are characterised by their large diameter  
77 (2-17 mm) and their jet-black pigmented disc. In cross-section, underneath the black epithecium, asci  
78 containing spores are present in the hymenium, covering the algal layer (Fig. 1b). The thallus in cross-  
79 section shows a very thin cortex, protecting a discontinuous algal layer. The central axis of *U. taylorii*  
80 is divided into several smaller axial strands by the protruding medullae, which are surrounded by some  
81 algae. Between these axial strands, lax medulla is observed (Fig. 1c).

82

#### 83 2.1.2. Chemical characterisation of *Usnea taylorii*

84 The extraction yields with acetone ranged between 0.5 and 1.0% of specialised metabolites for the six  
85 replicates of dried lichen materials with a mean value of  $0.8 \pm 0.2\%$ . Preliminary experiments showed  
86 that more than 95% of the specialised compounds were extracted from the lichen *U. taylorii* by rinsing

87 intact air-dry thalli in acetone. Thalli had only one or two main specialised metabolites. The  
88 concentration of usnic acid ranged from 2.5 to 5.4 mg·g<sup>-1</sup> dry mass (DM) of lichen, with a mean value  
89 ± s.d. of 4.1 ± 1.1 mg·g<sup>-1</sup> DM (n = 6). Sugar and polyol profiling was also performed. A low diversity was  
90 observed, with only seven metabolites quantified. Arabitol was the most important polyol, reaching  
91 138.4 ± 25.8 mg·g<sup>-1</sup> DM (mean value ± s.d., Table S1, Fig 2).

92 Preliminary LDI-MS analysis of an acetone extract of *Usnea taylorii* confirmed that the (+)-usnic  
93 acid was the only specialised metabolite detected through its deprotonated molecule (*m/z* 343) along  
94 with a fragment ion at *m/z* 329 ([M-Me]<sup>-</sup>) (Fig. S1). Usnic acid was consequently imaged through the  
95 *m/z* 343 ion. D-arabitol, the major polyol quantified by GC, was also imaged by mass spectrometry.  
96 However, because D-arabitol does not absorb at the wavelength of the laser used for LDI-MSI, a MALDI  
97 matrix solution was sprayed on *U. taylorii* slices and images were obtained using MALDI-MSI (through  
98 its sodium adduct observed at *m/z* 175).

99 *In situ* LDI-MSI experiments applied to a slice of *Usnea taylorii*'s branch revealed that usnic acid  
100 was located in the peripheral layer of the thallus, *i.e.*, in the cortex and in the uppermost parts of the  
101 medulla including the photobiont layer (Fig. 3a). Likewise, usnic acid was allocated to the external  
102 layers of the apothecium (epithecium, hymenium and the underside of the apothecium) (Fig. 3c). D-  
103 arabitol was present mainly in the lax medulla, the cortex and the algal layer (Fig. 4a), but occurred in  
104 lower intensities in the axial strands of the lichen branches. In apothecia, the signal corresponding to  
105 D-arabitol was stronger in the layer containing the lax medulla and algae than in the external layers of  
106 apothecium (epithecium and hymenium) (Fig. 4b). To perform a comparison between lichen parts  
107 eaten by snails or not, some branches and apothecia were sliced and analysed by LDI-MSI. Snails  
108 consumed parts of the cortex, the algal layer and the lax medulla of the branches. In apothecia,  
109 epithecium, hymenium as well as the underside of the apothecium were grazed. All these tissues  
110 contain usnic acid (Fig. 3b and 3d) and D-arabitol (Fig. 4).

111

## 112 2.2. Snail behaviour and lichen consumption experiments

### 113 2.2.1. Feeding choice arenas

114 There was no site effect on the distribution of snails in the feeding choice arenas ( $\text{Chi}^2 = 0.397$ ,  $\text{df} = 2$ ,  
115  $P = 0.820$ ). During the nutritional phase, 67.9% ( $\pm \text{se} = 4.11\%$ ) of the snails preferred to stay near the  
116 rock shelter (rock versus intact lichen  $P < 0.001$ , rock versus rinsed lichen  $P < 0.001$ ), while the rest of  
117 the snails were equally distributed ( $P = 0.70$ ) in arms with intact ( $16.7 \pm 2.5\%$ ) and rinsed lichens ( $15.5$   
118  $\pm 3.2\%$ ) (Fig. 5).

119 The removal of acetone-soluble metabolites did not promote lichen consumption (mean  $\pm$  s.e.  
120 =  $0.38 \pm 0.12 \mu\text{m}^2/\text{snail}$ ; BRA =  $0.25 \pm 0.11 \mu\text{m}^2/\text{snail}$ ; MAS =  $0.50 \pm 0.21 \mu\text{m}^2/\text{snail}$ ). No differences  
121 were detected among treatments ( $\text{Chi}^2 = 0.874$ ,  $\text{df} = 1$ ,  $P = 0.350$ ) and collecting sites ( $\text{Chi}^2 = 1.553$ ,  $\text{df}$

122 = 1,  $P = 0.213$ ). Morphological analyses of the consumed parts showed distinct marks of radula on the  
123 cortex, the algal layer and the lax medulla of the branches, while the axial strands of branches always  
124 remained intact (Fig. 6).

125

#### 126 2.2.2.No-choice experiments

127 Tested metabolites (usnic acid and/or arabitol) were added to a starch gel at the mean concentrations  
128 found in the thalli. The feeding experiment lasted 48 hours. Gel-mixed metabolites had a significant  
129 impact on their consumption by snails ( $\text{Chi}^2 = 57.47$ ,  $\text{df} = 3$ ,  $P < 0.001$ ; Fig. 7). Snails offered gels with  
130 usnic acid alone mainly avoided it (Tukey,  $P < 0.001$ ). Gels supplemented with arabitol were eaten as  
131 much as the starch control alone (Tukey,  $P = 0.795$ ; Fig. 7). Gels containing a mixture of arabitol and  
132 usnic acid were consumed more than usnic acid gels alone (Tukey,  $P < 0.001$ ), but less than arabitol  
133 gels alone (Tukey,  $P = 0.009$ ).

134

### 135 3. Discussion

136 The fruticose lichen *U. taylorii* (Parmeliaceae), found in nutrient-poor environments, was expected to  
137 be increasingly grazed by the snail *N. hookeri*, after acetone rinsing. Indeed, Gauslaa (2005) evidenced  
138 that acetone-rinsed lichens belonging to Parmeliaceae, rich in specialised metabolites, were more  
139 palatable to generalist land snails. Contrary to our expectation snails were equally distributed between  
140 rinsed and intact thalli in the four arms of the feeding choice arenas. Most of the snails were counted  
141 on the rocks in the two other arms, suggesting that *U. taylorii* could not be a resting place after feeding.  
142 Although information about lichen choice was lost because 68% of the snails preferred the rocks,  
143 simulations made from the real data set confirmed that absence of difference was not a bias due to a  
144 very low power of the test (Table S2). Lichen consumption was not significantly different between  
145 treatments. Thus, removal of acetone-soluble metabolites of *U. taylorii* did not increase lichen  
146 consumption by the snail. This result is consistent with the results of Asplund (2010), who observed  
147 that usnic acid did not affect snail grazing on the lichen *Lobaria scrobiculata*. In contrast, Asplund  
148 (2011b) described snail grazing (*Cochlodina laminata*) in depth after acetone rinsing of two *Lobaria*  
149 species, while the snail avoided the medulla before removal of acetone-soluble metabolites. However,  
150 *Lobaria pulmonaria* did not contain usnic acid (Asplund et al., 2018), suggesting that other compounds  
151 (stictic acid derivatives) were deterrent. According to the study of Boch et al. (2015) only one of three  
152 lichen species containing at least usnic acid showed chemical snail defence. From a relationship with  
153 lichen frequency in field, the authors deduced that the more the lichen was frequent, the higher was  
154 the consumption by snails. This relationship cannot be the rule in the present study since *U. taylorii*  
155 was absent from Branca site. By a deficit in specialised metabolites and a low concentration in usnic  
156 acid ( $4.1 \text{ mg.g}^{-1}$ ), *U. taylorii* appeared closer to the lichens belonging to Physciaceae and Teloschistales

157 described by Gauslaa (2005) although common in nutrient-rich environments. In these lichens, the  
158 author suggested that the specialised metabolites could protect thalli against excess light but could  
159 not deter grazing. In line with this, usnic acid is known to provide photoprotection to the algal cells  
160 (Nybakken and Gauslaa, 2007) and regulate their development (Bačkor et al., 2010). Gadea et al. (2017)  
161 showed that usnic acid was retrieved from faeces of *N. hookeri* after *U. taylorii* intake, meaning that  
162 the snail excreted the metabolite, but that did not mean that usnic acid was appetent and/or not toxic  
163 for the snail.

164 None of the studies cited above considered primary metabolites as feeding stimulants. For this  
165 reason, isolated compounds were tested separately and in mixture, in no-choice experiments.  
166 Usnic acid deterred *N. hookeri* when extracted and incorporated in a waxy starch gel, while it was  
167 consumed in lichen thalli. In contrast, Asplund et al. (2010) observed that filter paper added with  
168 usnic acid from the lichen *Lobaria scrobiculata* did not affect *Cepaea hortensis* grazing, despite the  
169 high content used (47.6 mg.g<sup>-1</sup>). Filter paper, made of cellulose and known to be highly attractive  
170 to helioid snails, could be much more palatable than waxy starch was to *N. hookeri*. In the present  
171 study, the repellent effect of pure usnic acid in the starch gel was mitigated by the addition of D-  
172 arabitol. The phagostimulant effect of D-arabitol for the snail has already been observed when the  
173 snail fed on the lichen *Argopsis friesiana* (Gadea et al., 2018). In a similar way, sucrose increased  
174 the amount of agar baits ingested by the slug *Deroceras reticulatum*, more than glucose, lactose  
175 and fructose (Henderson et al., 1992). These studies highlight the key role of primary metabolites  
176 for phytophages. Polyols are attractive for snails as nutrient metabolites that provide energy after  
177 oxidative conversion and help produce mucus, which enables body hydration, locomotion, and  
178 food foraging (Ng et al., 2013). In sub-Arctic lichens, polyol leaching was positively correlated with  
179 polyol concentration and in wet conditions (after a rain event, for example, which is so common  
180 in the sub-Antarctic), lichens lose up to 10% of their polyols through leaching (Dudley and  
181 Lechowicz, 1987). Consequently, snails might be first attracted by the exudate of arabitol, licking  
182 it and then would start grazing the cortex containing usnic acid. In contrast, the gustatory receptors  
183 of the snails (i.e. buccal lips and anterior tentacles) were in direct contact with usnic acid when the  
184 metabolite was incorporated to starch gels, thus revealing its role in chemical snail defence. Mass  
185 spectrometry imaging of the two metabolites gave arguments in support of the snail feeding  
186 pattern in the field. Spatial mapping revealed that arabitol and usnic acid were found partly  
187 collocated in the cortex and in the apothecia. Accordingly, the snails preferred to graze the  
188 apothecia, the lax medulla and the cortex that contained both fungal mycelium and algae with  
189 usnic acid and arabitol. In addition to arabitol leaching, the high concentration of arabitol in *U.*  
190 *taylorii*, thirty times as much as usnic acid, might explain why the feeding stimulus “arabitol” was  
191 stronger than the repellent stimulus “usnic acid”. This case study reveals that, as in plant-

192 phytophage interactions (Jamieson et al., 2017), primary metabolites are able to overcome  
193 deterrent metabolites and should be considered as important as specialised metabolites in lichen-  
194 lichenophage interactions.

195

#### 196 4. Conclusions

197 High amounts of arabitol, useful for lichen survival in cold places, makes *Usnea taylorii* vulnerable to  
198 grazing by a generalist gastropod, despite the production of a deterrent compound, namely, usnic acid.  
199 In addition to classical feeding experiments, the mass spectrometry imaging of deterrent and attractive  
200 compounds improved our knowledge of lichen/snail trophic interactions. Although snails are often  
201 classified as opportunistic feeders, their nutritional activity appears governed by the chemical quality  
202 of the food and primarily by nutrient availability. This lichen/snail case study could be applied to other  
203 biological models, for instance, terrestrial gastropods well known as crop pests in agriculture,  
204 horticulture and orchards.

205

#### 206 5. Experimental

##### 207 5.1. *Snail Collection and Lichen Material*

208 Adult individuals of *Notodiscus hookeri* Reeve (Charopidae) were collected on Possession Island during  
209 Austral summer in November 2015. Information about climate and topography is reported in Charrier  
210 et al. (2013). Snails came from two fell-fields, Mont Branca (BRA, 46°26'5.61"S; 51°50'10.68"E, 200 m  
211 - 383 m) and Mascarin Summit (MAS, 46°26'10.09" S; 51°45'20.58" E, 600 m - 930 m) (a map of the  
212 sampling sites is available in Gadea et al. (2018)).

213 The fruticose chlorolichen *Usnea taylorii* Hook. f. & Taylor (Parmeliaceae), encountered at MAS in the  
214 snail habitat, was harvested on Possession Island during Austral summer in November 2015. It was  
215 identified by Dr Damien Ertz and confirmed by Dr Philippe Clerc. Voucher specimens were deposited  
216 at the herbarium of the Faculty of Pharmacy of Rennes 1, Department of Pharmacognosy and  
217 Mycology, under the reference REN000141.

218

##### 219 5.2. *Macroscopic and microscopic analysis of Usnea taylorii*

220 Thallus morphology was examined using a dissecting microscope Leica MZ6 (Leica Microsystems  
221 GmbH, Wetzlar, Germany). Anatomical observations were made using a Leica DM2000 LED microscope  
222 (Leica Microsystems GmbH, Wetzlar, Germany). Longitudinal sections of apothecia and cross-sections  
223 of thalli were made using a hand-razor blade, and longitudinal sections of thalli were cut from the well-  
224 developed thicker branches. The ratio “% of axis thickness/% of medulla thickness” = ratio A/M, which  
225 is a good discriminator of *Usnea* species (Truong et al., 2011), was calculated. K (potassium hydroxide)

226 and P (*para*-phenylenediamine) spot tests according to Hale (1979) were directly applied to the  
227 medulla on longitudinal sections of the branches.

228

229 5.3. HPLC-DAD-MS analysis of specialised metabolites

230 5.3.1. Instrumental settings

231 HPLC separation and quantification of usnic acid and *Usnea taylorii* extracts were performed on a  
232 Prominence Shimadzu HPLC system (Marne La Vallée, France) equipped with a Kinetex C<sub>18</sub> HPLC  
233 column (100 x 4.6 mm, 2.6 µm, 6A, Phenomenex, Torrance, CA, USA). The HPLC system comprises a  
234 quaternary pump (LC20ASDP), a surveyor autosampler (SIL-20AHT) and a diode array detector (SPD-  
235 M20A). The separation was achieved using an acidic water/acetonitrile system as previously described  
236 (Gadea et al., 2017). The ESI-mass spectra were obtained from an Expression Advion CMS apparatus  
237 (Advion, Ithaca, USA) The mass spectra were recorded in the negative-ion mode in a mass range of 100  
238 to 1200 Da, applying the same parameters previously described by Gadea et al. (2017). The spectral  
239 data from the photodiode array detector were collected 48 min over the 200-500 nm range of the  
240 absorption spectrum, and the chromatograms were plotted at the maximum wavelength of absorption  
241 ( $\lambda_{max}$ ) of the main metabolites. Peaks were assigned according to the retention time, UV spectra and  
242 mass spectra. The same chromatographic method was applied for the standards and the samples.

243

244 5.3.2. Sample and standard preparation

245 Specialised metabolites of six specimens of the whole fertile thallus of *Usnea taylorii* (642 to 1000 mg)  
246 were extracted by the acetone rinsing method, three times (5 mL, 20 min), at room temperature and  
247 were air-dried until the solvent had evaporated (Solhaug and Gauslaa, 2001). Acetone extracts (N=6)  
248 were used for the chemical analysis while the rinsed lichen thalli were kept for the feeding choice  
249 experiments. The yield, defined as the ratio between the masses of the extracts and the dry lichens,  
250 was calculated for each extraction. All extracts obtained from *Usnea taylorii* were dissolved in bi-  
251 distilled tetrahydrofuran at the concentration of 0.5 g·L<sup>-1</sup>. Then, the extracts were filtered through a  
252 Nylon syringe F2504-1 (Thermo Scientific, Rockwood, USA, 0.45 µm x 4 mm) and transferred to an  
253 appropriate vial for automatic injection of 10 µL aliquot into the HPLC system.

254

255 5.3.3. Analytical method validation

256 All validation parameters were determined following the International Conference on Harmonization  
257 (ICH) Guidelines (ICH, 2005) using HPLC analyses, and the following characteristics were evaluated:  
258 linearity, limits of detection (LOD) and quantification (LOQ), repeatability inter-day and intra-day. Four  
259 stock standard solutions of (+)-usnic acid (Sigma-Aldrich; 329967) were prepared by dissolving  
260 approximately 2 mg in 4 mL tetrahydrofurane to reach a concentration of 0.5 mg·mL<sup>-1</sup>. Then, six

261 working standard solutions of usnic acid were prepared by appropriate dilution of each stock solution  
262 with acetonitrile to generate concentrations ranging from 0.07 to 0.09 mg.mL<sup>-1</sup> for the external  
263 standard calibration curve and the determination of the regression line. Hence, the concentrations of  
264 usnic acid in *U. taylorii* were calculated, based on peak areas. The limits of detection and quantification  
265 were determined from the y-intercept standard deviation and the slope of the calibration curve. For  
266 the calculation of the intra-day repeatability, a dilution of usnic acid (0.05 mg.mL<sup>-1</sup>) was injected six  
267 times the same day. These assays were repeated on four different days for inter-day repeatability. The  
268 coefficient of variation and standard deviation were then calculated. Coefficients of variation of less  
269 than 10% for intra-day and for inter-day were accepted. The results of the validation are available in  
270 Table 1. Concentrations were obtained on the basis of calibration curves and were expressed in mg.g<sup>-1</sup>  
271 <sup>1</sup> DM of the lichen part.

272

#### 273 5.4. Sugar and polyol profiling

274 Sugars and polyols were extracted from *U. taylorii* entire fertile thalli (N=6) and profiled by gas  
275 chromatography-flame ionisation detector (GC-FID) (Thermo-Fisher Scientific, Waltham, CA, USA), as  
276 previously described by Gadea et al. (2018). Adonitol (20 mM) was used as internal standard. The  
277 metabolites were identified from their retention time by comparison with external standards.  
278 Concentrations were obtained on the basis of internal standards and were expressed in mg.g<sup>-1</sup> DM of  
279 the lichen part.

280

#### 281 5.5. Mass Spectrometry Imaging (MSI)

282 *Usnea taylorii* samples were hand-cut using a razor blade to afford slices approximately 100 µm thick.  
283 Branches and apothecia slices were fixed on a carbon-conductive adhesive tape that was, in turn, fixed  
284 on an indium tin oxide (ITO) slide (Bruker Daltonics, Bremen, Germany, cat. no. 237001). For laser  
285 desorption ionisation (LDI)-MSI measurement, no further preparation step was required. For matrix -  
286 assisted laser desorption Ionisation (MALDI)-MSI measurements, DHB matrix solution (50 mg.mL<sup>-1</sup> in  
287 50% methanol) was homogeneously applied with a custom-designed spraying robot.

288 All MSI measurements were performed using an Autoflex-Speed MALDI-TOF/TOF spectrometer  
289 (Bruker Daltonics, Bremen, Germany) equipped with a Smartbeam laser (355 nm, 1000 Hz) and  
290 controlled using the Flex Control 3.4 software package. The mass spectrometer was used in the  
291 reflectron mode with a negative polarity for LDI-MSI and a positive polarity for MALDI-MSI. Spectra  
292 were acquired in the mass range of m/z 100–600 for all (x, y) coordinates corresponding to the imaged  
293 tissue.

294 The laser raster size was set at 35 microns. The signal was initially optimised by manually  
295 adjusting the laser power and the number of laser shots fired. Accordingly, full-scan MS experiments

296 were run by accumulating 400 laser shots per raster position and by using the laser power leading to  
297 the best signal-to-noise ratio. Image acquisition was performed using the Flex Imaging 4.0 (Bruker  
298 Daltonics) software package. The correlation of the target plate with the optical image was obtained  
299 from three distinct teaching points following the procedure of the Flex Imaging software (Bruker  
300 Daltonics).

301

## 302 5.6. Snail behaviour experiments

### 303 5.6.1. Snail feeding choice arena

304 Each fertile thallus of *U. taylorii* was divided into two pieces of equal size, always containing a  
305 branch with the terminal apothecia. Half of these thalli were randomly taken to extract the specialised  
306 metabolites and constituted the rinsed lichens ("Rinsed"). The other group contained intact lichens  
307 ("Intact"). Residual acetone was evaporated for at least 20 hours before the experiment.

308 One hundred and sixty-eight *N. hookeri* per site were divided randomly into eight subgroups of 21  
309 snails. No significant differences in shell size were observed among snail subgroups (mean  $\pm$  s.d. = 4.80  
310  $\pm$  0.37; ANOVA,  $F = 1.590$ ,  $df = 7$ ,  $P = 0.137$ ). Two days before the experiment, snails were starved to  
311 enhance their feeding motivation. The snails were placed inside a feeding choice arena with six arms,  
312 made of plexiglass (Plast'it supplier, Rennes, France, under the concept proposed by Dr. Maryvonne  
313 Charrier, Fig. 8). Wet synthetic foam was placed at the bottom of each arena to maintain strong  
314 humidity necessary for snail activity. Similar lichen samples were placed at both ends in opposite arms,  
315 giving the choice between two: two "Intact" and two "Rinsed" samples. To avoid a stressful condition  
316 for snails used to aggregate under a shelter (Dahirel et al., 2018), the two latter arms contained a small  
317 bare rock each, representing secure refuges during resting phases. The experiment was replicated  
318 eight times per site. Each feeding choice arena received 21 snails and was maintained in a climatic  
319 chamber under a light/dark photoperiod set as follows: 10 °C day (12 hours) and 6 °C night (12 hours).  
320 A rotation of the arenas was done four times a day to avoid a position effect in the climatic chamber.  
321 Experiments started at the beginning of the nocturnal phase and ended 48 hours after.

322 To analyse snail distribution, the position of each snail in the arena was reported. To evaluate lichen  
323 consumption, radula marks on lichen thalli were observed under stereomicroscope (Zeiss, France). The  
324 area of radula impacts was measured (in  $\mu\text{m}^2/\text{snail}$ ) with Labscope software (version 2.0, Zeiss, France).

325

### 326 5.6.2. No-choice experiments on isolated compounds

327 Two hundred and forty adult snails were separated into four subgroups: one control and three tested  
328 groups of 60 snails each (usnic acid, usnic acid + arabitol and arabitol). No significant difference in shell  
329 size was observed among subgroups (mean  $\pm$  s.d. = 4.81  $\pm$  0.33 mm; ANOVA,  $F=2.472$ ,  $df=3$ ,  $P = 0.062$ ).  
330 No-choice experiments were performed in two-compartment Petri dishes (Greiner Bio One, Austria).

331 Wet nonwoven sterile gauze was placed in each compartment to maintain high humidity. Waxy starch  
332 gels were prepared at the concentration of 15% and small discs (diameter: 5 mm, thickness 2 mm)  
333 were manufactured using a custom-designed device (Plast'it supplier, Rennes, France, under the  
334 concept proposed by Dr. Maryvonne Charrier). Metabolites (usnic acid, arabitol or a mixture of usnic  
335 acid and arabitol) were added to the gel at the same concentrations as those quantified in the entire  
336 fertile thalli ( $4 \text{ mg}\cdot\text{g}^{-1}$  for usnic acid and  $100 \text{ mg}\cdot\text{g}^{-1}$  for arabitol). Waxy starch gels alone were considered  
337 as positive controls. Snails had no choice, either facing a gel containing metabolite or a control gel, for  
338 48 hours. At the end of the experiment, gels were photographed, and a feeding score was estimated.  
339 To calculate the feeding score, a calibrated grid placed on the gel provided the area consumed, then a  
340 correction factor was applied (x1 for consumption of the surface layer and x2 when the gel was  
341 consumed in its full thickness; Fig. S2).

342

### 343 5.6.3. Statistical analyses

344 Snail distribution in the arenas was analysed by a multidimensional logistic model for proportion data.  
345 The effect of the site (BRA versus MAS) was tested using a likelihood ratio test on a first model. Since  
346 the site did not have a significant effect, a second model was built without any independent variable,  
347 that allowed the comparison of the proportion of individuals choosing each treatment (intact lichen,  
348 rinsed lichen and rock) using a series of Wald tests where p-values were corrected using the false  
349 discovery rate method. To analyse lichen consumption (areas in  $\mu\text{m}^2$ ) according to the treatment  
350 (intact versus rinsed) and the snail sampling site, a Wald test was applied on a GLMM (family: quasi,  
351 link: identity, variance:  $V = \mu$ ).

352 In the no-choice experiments, to determine if metabolites were differently consumed  
353 compared to the control, a likelihood ratio test was used on a generalised linear model (GLM,  
354 distribution: negative binomial, link function: log), in which the snail sampling site was introduced as a  
355 fixed factor. Tukey's post hoc pairwise comparisons were realised. As no differences between sampling  
356 sites were observed on the feeding score ( $\chi^2 = 0.69$ ,  $df = 1$ ,  $P = 0.408$ ), the results of both sampling sites  
357 were combined. All statistical analyses were made using R software V. 3.4.3 (R Core Team, 2017). The  
358 R packages used were: car, emmeans, lme4, nnet, MASS, MuMin and RVAideMemoire.

359

### 360 *Acknowledgments*

361 Subantarctic field trip was funded by l'Institut Polaire Paul-Émile Victor, Plouzané, France (IPEV,  
362 programme 136). Dr Damien Ertz is acknowledged for the identification on the field of the lichen *Usnea*  
363 *taylorii* and for his help with the snails' collection. Julien Tommasino and Grichka Biver are thanked for  
364 their contribution in producing a part of this data set. Aude Boutet, Julien Tommasino and Benjamin  
365 Ferlay are warmly thanked for their help during the fieldwork. Sugars and polyols quantifications were

366 conducted at the P2M2 Platform, thanks to Dr Alain Bouchereau, and with the technical assistance of  
367 Catherine Jonard and Nathalie Marnet, which are warmly thanked. Authors are also indebted to Dr  
368 Maxime Dahirel for his advices in statistics and to Dr Maxime Hervé who wrote the R script that  
369 referred to feeding choice experiments (Link to his page: [https://www.maximeherve.com/r-et-](https://www.maximeherve.com/r-et-statistiques)  
370 [statistiques](https://www.maximeherve.com/r-et-statistiques), and package 'RVAideMemoire'). We thank Dr Béatrice Legouin for her guidance on the  
371 analytical method validation. We are grateful to the anonymous referees for their useful comments  
372 that contributed to improve the manuscript.

373

#### 374 *Ethical Statement*

375 The French Polar Institute (IPEV) is the authority that supported this research based on the advice of  
376 its scientific council. The sites visited during this study did not require any access authorization. All  
377 research and data reported here were obtained in compliance with all current French laws. Collecting  
378 and transport of specimens of the species *Notodiscus hookeri* were authorized by the Prefect of Ille-  
379 et-Vilaine, France, licence N°35–120 delivered in October 2015.

380

#### 381 *Figures and Legends*

382

383 Fig. 1. Morphological description of *U. taylorii*. (a) *U. taylorii* entire thalli. Insets correspond to the  
384 magnification used in (b) for the apothecia and in (c) for the branch. (b) Apothecium in cross-section;  
385 (c) branches in cross and longitudinal sections. AL = algal layer; AS = axial strands; C = cortical layer and  
386 E = epithecium, HM = hymenium containing asci and spores; HP = hypothecium; LM = lax medulla;  
387 UA=underside of the apothecium. In colour online.

388

389 Fig. 2. Chemical structures of the two main metabolites of *Usnea taylorii*: the specialised metabolite  
390 (+)-usnic acid and the primary metabolite D-arabitol.

391

392 Fig. 3. Distribution of usnic acid ( $[M-H]^-$  ion;  $m/z$  343) in samples of *U. taylorii*. a. Intact branch; b.  
393 Grazed branch (cross-sections); c. Intact apothecium, d. Grazed apothecium (longitudinal sections).  
394 Each panel features side by side the optical image of the lichen section (left side) and the mass  
395 spectrometry imaging results (right side). The grazing marks were highlighted by blue arrows. Intensity  
396 scale was adjusted to maximise the visualisation of usnic acid. In colour online.

397

398 Fig. 4. Distribution of arabitol ( $[M+Na]^+$  ion;  $m/z$  175) in samples of *U. taylorii*. a. Grazed branches; b.  
399 Intact apothecium. On each panel are side by side the optical image of the lichen section (left side) and  
400 the mass spectrometry imaging results (right side). The grazing marks on the branches were

401 highlighted by a blue arrow. Intensity scale was adjusted to maximise differences in segregation of  
402 arabitol. In colour online.

403

404 Fig. 5. Snail distribution (means + s.e., N= 21 snails x 8 arenas = replicates x 2 sites) in the arms of  
405 feeding choice arenas (three-test choice with two identical opposite arms), during the nutritional  
406 phase. The letters *a* and *b* indicate significant differences between arena arms containing lichens and  
407 arms containing the little rocks.

408

409 Fig. 6. Snails ate the apothecium (a) and the external layers of branches (b). Arrows designate snail  
410 grazing marks. In colour online.

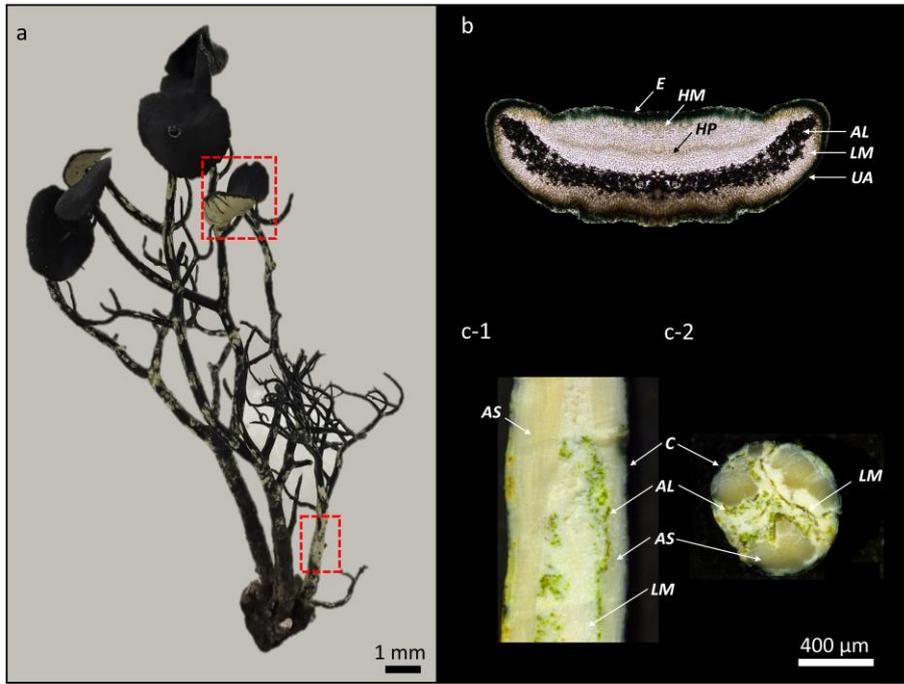
411

412 Fig. 7. Gel consumption (Mean  $\pm$  s.e.) by the snails (N=240), according to the metabolite tested on  
413 starch gel. Gel consumption was estimated by a feeding score. The positive control was the starch gel  
414 without metabolites. The lowercase superscript letters a, b and c indicate significant differences  
415 between positive control and usnic acid containing gels (alone or in mixture with arabitol). Gels with  
416 arabitol were not significantly different from the positive control.

417

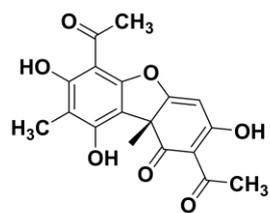
418 Fig. 8. Choice arena device. Lichen samples, intact or acetone rinsed, and rocks were arranged in  
419 opposite arms. Snails (N=21 x 8 arenas x 2 sites) were placed in the central area at the beginning of the  
420 experiment and 48 hours later, snail distribution and lichen consumption were analysed.

421



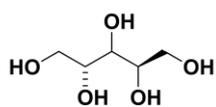
422  
423  
424

Revised manuscript



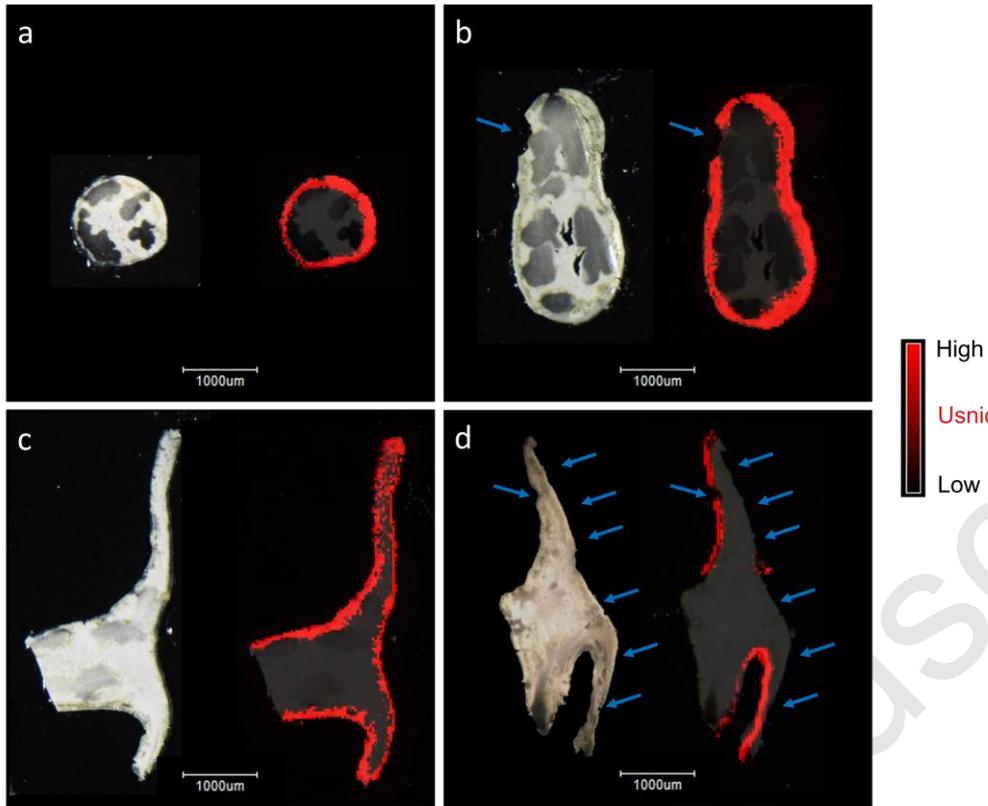
425

(+)-usnic acid



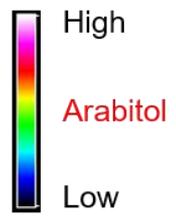
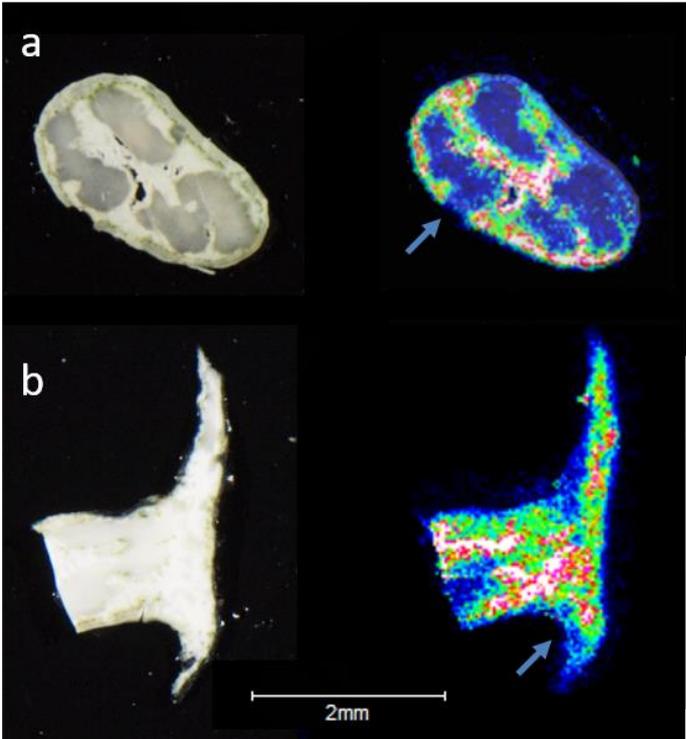
D-arabitol

Revised manuscript



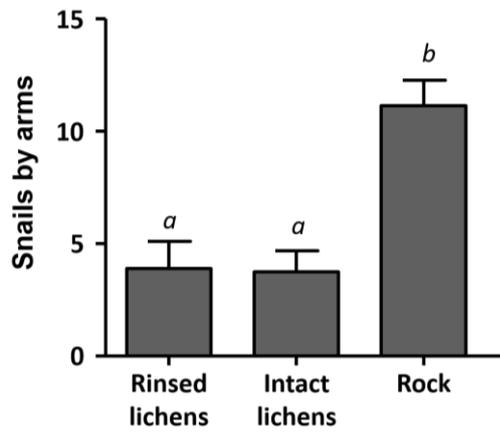
426

427



428  
429

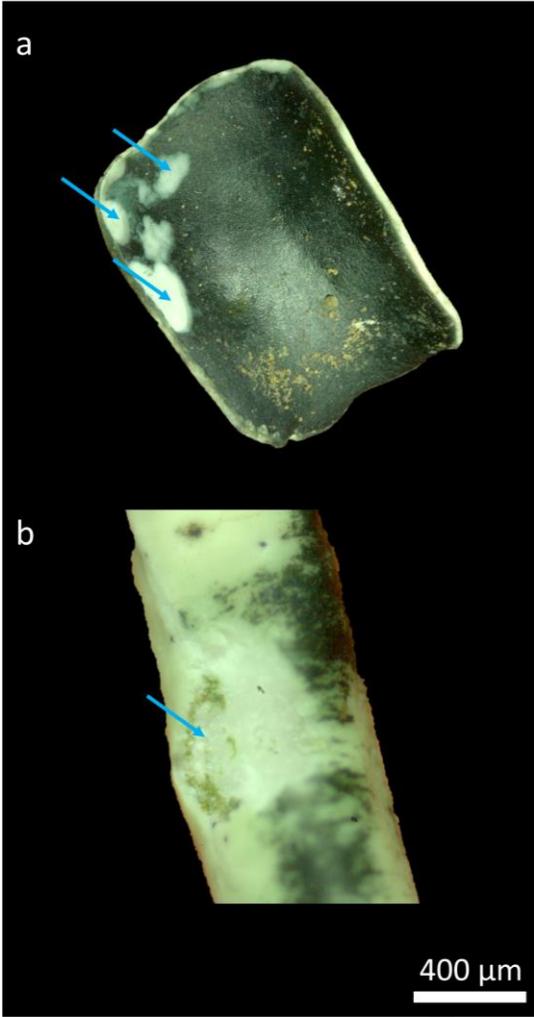
Revised manuscript



430

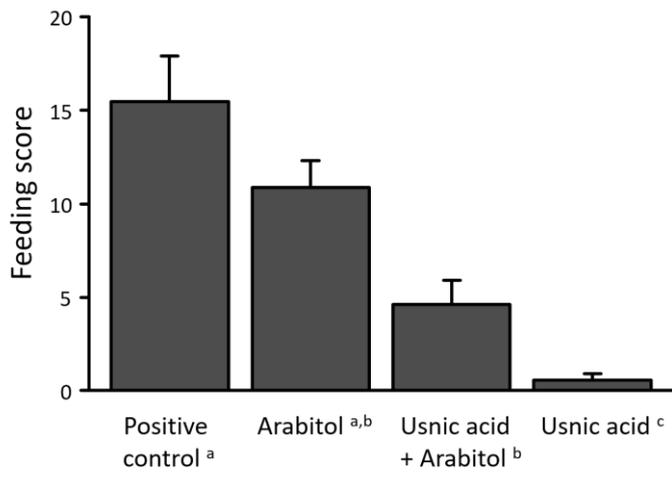
431

Revised manuscript



432

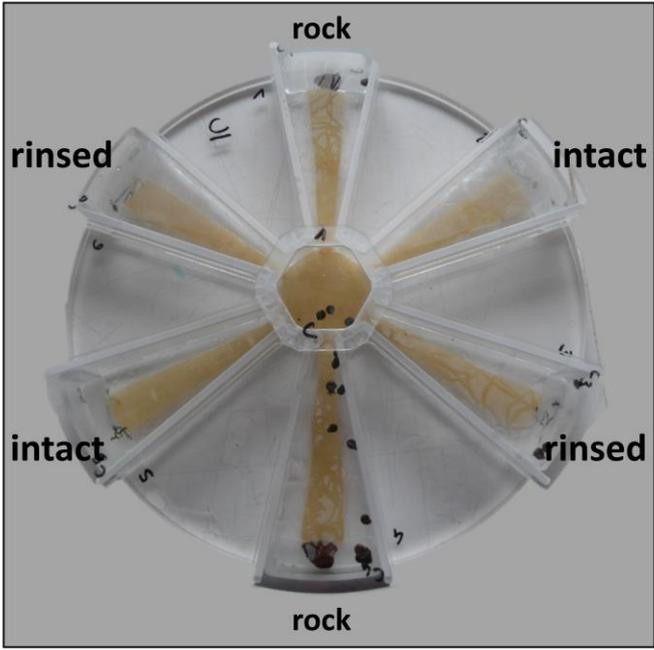
433



434

435

Revised manuscript



436

Revised manuscript

437 *Tables*

438

439 **Table 1:** Results of various parameters of validation studies for usnic acid quantification

440

Revised manuscript

Parameters	Usnic acid
Calibration curve equation	$y = 65613076x - 95165$
Correlation coefficient value ( $R^2$ )	0.9940
LOD ( $\text{mg}\cdot\text{mL}^{-1}$ )	0.0001
LOQ ( $\text{mg}\cdot\text{mL}^{-1}$ )	0.0070
Calibration range ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	0.0070 to 0.0900
Intraday precision (RSD, n=6)	less than 3%
Interday precision (RSD, n=24)	less than 2%

441 LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation

442 *References*

- 443 Asplund, J., 2011a. Chemical races of *Lobaria pulmonaria* differ in palatability to gastropods.  
444 *Lichenologist* 43, 491–494. <https://doi.org/10.1017/S0024282911000387>
- 445 Asplund, J., 2011b. Snails avoid the medulla of *Lobaria pulmonaria* and *L. scrobiculata* due to presence  
446 of secondary compounds. *Fungal Ecol* 4, 356–358.  
447 <https://doi.org/10.1016/j.funeco.2011.05.002>
- 448 Asplund, J., Solhaug, K.A., Gauslaa, Y., 2010. Optimal defense: snails avoid reproductive parts of the  
449 lichen *Lobaria scrobiculata* due to internal defense allocation. *Ecology* 91, 3100–3105.  
450 <https://doi.org/10.1890/09-1829.1>
- 451 Asplund, J., Strandin, O.V., Gauslaa, Y., 2018. Gastropod grazing of epiphytic lichen-dominated  
452 communities depends on tree species. *Basic and Applied Ecology* 32, 96–102.  
453 <https://doi.org/10.1016/j.baae.2018.07.007>
- 454 Asplund, J., Wardle, D.A., 2016. How lichens impact on terrestrial community and ecosystem  
455 properties. *Biol Rev.* <https://doi.org/10.1111/brv.12305>
- 456 Bačkor, M., Klemová, K., Bačkorová, M., Ivanova, V., 2010. Comparison of the phytotoxic effects of  
457 usnic acid on cultures of free-living alga *Scenedesmus quadricauda* and aposymbiotically  
458 grown lichen photobiont *Trebouxia erici*. *J Chem Ecol* 36, 405–411.  
459 <https://doi.org/10.1007/s10886-010-9776-4>
- 460 Benesperi, R., Tretiach, M., 2004. Differential land snail damage to selected species of the lichen genus  
461 *Peltigera*. *Biochem Syst Ecol* 32, 127–138. [https://doi.org/10.1016/S0305-1978\(03\)00141-8](https://doi.org/10.1016/S0305-1978(03)00141-8)
- 462 Boch, S., Fischer, M., Prati, D., 2015. To eat or not to eat—relationship of lichen herbivory by snails  
463 with secondary compounds and field frequency of lichens. *J Plant Ecol* 8, 642–650.  
464 <https://doi.org/10.1093/jpe/rtv005>
- 465 Černajová, I., Svoboda, D., 2014. Lichen compounds of common epiphytic Parmeliaceae species deter  
466 gastropods both in laboratory and in Central European temperate forests. *Fungal Ecol* 11, 8–  
467 16. <https://doi.org/10.1016/j.funeco.2014.03.004>
- 468 Charrier, M., Fonty, G., Gaillard-Martinie, B., Ainouche, K., Andant, G., 2006. Isolation and  
469 characterization of cultivable fermentative bacteria from the intestine of two edible snails,  
470 *Helix pomatia* and *Cornu aspersum* (Gastropoda: Pulmonata). *Biol Res* 39, 669–681.  
471 <https://doi.org/10.4067/S0716-97602006000500010>
- 472 Charrier, M., Marie, A., Guillaume, D., Bédouet, L., Le Lannic, J., Roiland, C., Berland, S., Pierre, J.-S., Le  
473 Floch, M., Frenot, Y., Lebouvier, M., 2013. Soil calcium availability influences shell  
474 ecophenotype formation in the Sub-Antarctic land snail, *Notodiscus hookeri*. *PLoS ONE* 8.  
475 <https://doi.org/10.1371/journal.pone.0084527>

476 Charrier, M., Rouland, C., 2001. Mannan-degrading enzymes purified from the crop of the brown  
477 garden snail *Helix aspersa* Müller (Gastropoda Pulmonata). J Exp Zool 290, 125–135.  
478 <https://doi.org/10.1002/jez.1042>

479 Clark, S.J., Doods, C.J., Henderson, I.F., Martin, A.P., 1997. A bioassay for screening materials  
480 influencing feeding in the field slug *Deroceras reticulatum* (Müller) (Mollusca: Pulmonata). Ann  
481 Appl Biol 130, 379–385. <https://doi.org/10.1111/j.1744-7348.1997.tb06841.x>

482 Dudley, S.A., Lechowicz, M.J., 1987. Losses of polyol through leaching in Subarctic lichens. Plant Physiol  
483 83, 813–815.

484 Emmerich, R., Giez, I., Lange, O.L., Proksch, P., 1993. Toxicity and antifeedant activity of lichen  
485 compounds against the polyphagous herbivorous insect *Spodoptera littoralis*. Phytochemistry  
486 33, 1389–1394. [https://doi.org/10.1016/0031-9422\(93\)85097-B](https://doi.org/10.1016/0031-9422(93)85097-B)

487 Flari, V., Matoub, M., Rouland, C., 1995. Purification and characterization of a  $\beta$ -mannanase from the  
488 digestive tract of the edible snail *Helix lucorum* L. Carbohyd Res 275, 207–213.  
489 [https://doi.org/10.1016/0008-6215\(95\)00136-H](https://doi.org/10.1016/0008-6215(95)00136-H)

490 Fröberg, L., Stoll, P., Baur, A., Baur, B., 2011. Snail herbivory decreases cyanobacterial abundance and  
491 lichen diversity along cracks of limestone pavements. Ecosphere 2, art38.  
492 <https://doi.org/10.1890/ES10-00197.1>

493 Gadea, A., Le Lamer, A.-C., Le Gall, S., Jonard, C., Ferron, S., Catheline, D., Ertz, D., Le Pogam, P., Boustie,  
494 J., Lohézic-Le Dévéhat, F., Charrier, M., 2018. Intrathalline metabolite profiles in the lichen  
495 *Argopsis friesiana* shape Gastropod grazing patterns. J Chem Ecol 44, 471–482.  
496 <https://doi.org/10.1007/s10886-018-0953-1>

497 Gadea, A., Le Pogam, P., Biver, G., Boustie, J., Le Lamer, A.-C., Le Dévéhat, F., Charrier, M., 2017. Which  
498 specialized metabolites does the native Subantarctic Gastropod *Notodiscus hookeri* extract  
499 from the consumption of the lichens *Usnea taylorii* and *Pseudocyphellaria crocata*? Molecules  
500 22, 425. <https://doi.org/10.3390/molecules22030425>

501 Gauslaa, Y., 2005. Lichen palatability depends on investments in herbivore defence. Oecologia 143,  
502 94–105. <https://doi.org/10.1007/s00442-004-1768-z>

503 Goga, M., Pöykkö, H., Adlassnig, W., Bačkor, M., 2015. Response of the lichen-eating moth *Cleorodes*  
504 *lichenaria* larvae to varying amounts of usnic acid in the lichens. Arthropod Plant Interact 10,  
505 71–77. <https://doi.org/10.1007/s11829-015-9409-5>

506 Hale, M.E., 1979. How to know the lichens, 2nd ed. WC Brown Publishers, Dubuque, Iowa.

507 Henderson, I.F., Martin, A.P., Perry, J.N., 1992. Improving slug baits: the effects of some  
508 phagostimulants and molluscicides on ingestion by the slug, *Deroceras reticulatum* (Miiller)  
509 (Pulmonata: Limacidae). Ann Appl Biol 121, 423–430. <https://doi.org/10.1111/j.1744-7348.1992.tb03454.x>

510

511 Hesbacher, S., Baur, B., Baur, A., Proksch, P., 1995. Sequestration of lichen compounds by three species  
512 of terrestrial snails. *J Chem Ecol* 21, 233–246. <https://doi.org/10.1007/BF02036654>

513 Honegger, R., 1991. Functional aspects of the lichen symbiosis. *Annu Rev Plant Physiol Plant Mol Biol*  
514 42, 553–578. <https://doi.org/10.1146/annurev.pp.42.060191.003005>

515 Huneck, S., Yoshimura, I., 1996. Identification of lichen substances. Springer, Berlin ; New York.

516 ICH, 2005. Validation of analytical procedures: text and methodology. International Conference on  
517 Harmonization, IFPMA, Geneva, Switzerland.

518 Jamieson, M.A., Burkle, L.A., Manson, J.S., Runyon, J.B., Trowbridge, A.M., Zientek, J., 2017. Global  
519 change effects on plant–insect interactions: the role of phytochemistry. *Current Opinion in*  
520 *Insect Science, Global change biology \* Molecular physiology* 23, 70–80.  
521 <https://doi.org/10.1016/j.cois.2017.07.009>

522 Le Pogam, P., Legouin, B., Geairon, A., Rogniaux, H., Lohézic-Le Dévéhat, F., Obermayer, W., Boustie,  
523 J., Le Lamer, A.-C., 2016. Spatial mapping of lichen specialized metabolites using LDI-MSI:  
524 chemical ecology issues for *Ophioparma ventosa*. *Sci Rep* 6, 37807.  
525 <https://doi.org/10.1038/srep37807>

526 Liao, C., Piercey-Normore, M.D., Sorensen, J.L., Gough, K., 2010. *In situ* imaging of usnic acid in selected  
527 *Cladonia* spp. by vibrational spectroscopy. *Analyst* 135, 3242–3248.  
528 <https://doi.org/10.1039/COAN00533A>

529 Ng, T.P.T., Saltin, S.H., Davies, M.S., Johannesson, K., Stafford, R., Williams, G.A., 2013. Snails and their  
530 trails: the multiple functions of trail-following in gastropods. *Biol Rev* 88, 683–700.  
531 <https://doi.org/10.1111/brv.12023>

532 Nimis, P.L., Skert, N., 2006. Lichen chemistry and selective grazing by the coleopteran *Lasioderma*  
533 *serricornis*. *Environ Exp Bot* 55, 175–182. <https://doi.org/10.1016/j.envexpbot.2004.10.011>

534 Nybakken, L., Gauslaa, Y., 2007. Difference in secondary compounds and chlorophylls between fibrils  
535 and main stems in the lichen *Usnea longissima* suggests different functional roles.  
536 *Lichenologist* 39. <https://doi.org/10.1017/S0024282907007190>

537 Pöykkö, H., Hyvärinen, M., Bačkor, M., 2005. Removal of lichen secondary metabolites affects food  
538 choice and survival of lichenivorous moth larvae. *Ecology* 86, 2623–2632.  
539 <https://doi.org/10.1890/04-1632>

540 R Core Team, 2017. R: A language and environment for statistical computing. R Foundation for  
541 Statistical Computing, Vienna, Austria.

542 Roullier, C., Chollet-Krugler, M., Pferschy-Wenzig, E.-M., Maillard, A., Rechberger, G.N., Legouin-  
543 Gargadennec, B., Bauer, R., Boustie, J., 2011. Characterization and identification of  
544 mycosporines-like compounds in cyanolichens. Isolation of mycosporine hydroxyglutamicol

545 from *Nephroma laevigatum* Ach. *Phytochemistry* 72, 1348–1357.  
546 <https://doi.org/10.1016/j.phytochem.2011.04.002>

547 Solhaug, K.A., Gauslaa, Y., 2012. Secondary lichen compounds as protection against excess solar  
548 radiation and herbivores, in: Lüttge, U., Beyschlag, W., Büdel, B., Francis, D. (Eds.), *Progress in*  
549 *Botany*. Springer Berlin Heidelberg, pp. 283–304.

550 Solhaug, K.A., Gauslaa, Y., 2001. Acetone rinsing—a method for testing ecological and physiological roles  
551 of secondary compounds in living lichens. *Symbiosis* 30, 301–315.

552 Stocker-Wörgötter, E., 2008. Metabolic diversity of lichen-forming ascomycetous fungi: culturing,  
553 polyketide and shikimate metabolite production, and PKS genes. *Nat Prod Rep* 25, 188–200.  
554 <https://doi.org/10.1039/B606983P>

555 Truong, C., Bungartz, F., Clerc, P., 2011. The lichen genus *Usnea* (Parmeliaceae) in the tropical Andes  
556 and the Galapagos: species with a red-orange cortical or subcortical pigmentation. *bryologist*  
557 114, 477–504. <https://doi.org/10.1639/0007-2745-114.3.477>

558 Vatne, S., Solhøy, T., Asplund, J., Gauslaa, Y., 2010. Grazing damage in the old forest lichen *Lobaria*  
559 *pulmonaria* increases with gastropod abundance in deciduous forests. *Lichenologist* 42, 615–  
560 619. <https://doi.org/10.1017/S0024282910000356>

561