This is an author produced version of a paper published in FUNGAL ECOLOGY. This paper has been peer-reviewed and is proof-corrected, but does not include the journal pagination.

Citation for the published paper:
http://dx.doi.org/10.1016/j.funeco.2012.06.004

Access to the published version may require journal subscription.
Published with permission from: Elsevier

Standard set statement from the publisher:
What rights do I retain as a journal author*? the right to make copies (print or electronic) of the journal article for your own personal use, including for your own classroom teaching use; the right to make copies and distribute copies of the journal article (including via e-mail) to research colleagues, for personal use by such colleagues for scholarly purposes*; the right to post a pre-print version of the journal article on Internet websites including electronic pre-print servers, and to retain indefinitely such version on such servers or sites for scholarly purposes* (with some exceptions such as The Lancet and Cell Press. See also our information on electronic preprints for a more detailed discussion on these points)*; the right to post a revised personal version of the text of the final journal article (to reflect changes made in the peer review process) on your personal or institutional website or server for scholarly purposes*, incorporating the complete citation and with a link to the Digital Object Identifier (DOI) of the article (but not in subject-oriented or centralized repositories or institutional repositories with mandates for systematic postings unless there is a specific agreement with the publisher.); the right to present the journal article at a meeting or conference and to distribute copies of such paper or article to the delegates attending the meeting; for your employer, if the journal article is a ‘work for hire’, made within the scope of the author’s employment, the right to use all or part of the information in (any version of) the journal article for other intra-company use (e.g. training); patent and trademark rights and rights to any process or procedure described in the journal article; the right to include the journal article, in full or in part, in a thesis or dissertation; the right to use the journal article or any part thereof in a printed compilation of your works, such as collected writings or lecture notes (subsequent to publication of the article in the journal); and the right to prepare other derivative works, to extend the journal article into book-length form, or to otherwise re-use portions or excerpts in other works, with full acknowledgement of its original publication in the journal.
Epsilon Open Archive http://epsilon.slu.se
Contrasting changes in palatability following senescence of the lichenized fungi Lobaria pulmonaria and L. scrobiculata

Johan ASPLUND¹,²* and David A WARDLE¹

¹Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden.

²Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, NO-1432 Ås, Norway.

*Corresponding Author:

johan.asplund@slu.se

Phone: +47 47 357 127

Fax: +46 90 786 8163
Abstract (max 150 words):

Epiphytic lichens can contribute significantly to ecosystem nutrient input because they efficiently accumulate atmospheric mineral nutrients and, in the case of cyanolichens, also fix nitrogen. The rate at which carbon and other nutrients gained by lichens enters the ecosystem is determined by lichen litter decomposability and by invertebrate consumption of lichen litter. In turn, these processes are driven by the secondary compounds present in senesced lichens. Therefore, we explored how lichen palatability and concentrations of secondary compounds change with tissue senescence for *Lobaria pulmonaria*, a green algal lichen with cyanobacterial cephalodia, and *L. scrobiculata*, a cyanobacterial lichen. During senescence both lichens lost 38–48% of their stictic acid chemosyndrome, while *m*-scrobiculin and usnic acid in *L. scrobiculata* remained unchanged. Snails preferred senesced rather than fresh *L. pulmonaria*, while senesced *L. scrobiculata* were avoided. This provides evidence that species with labile secondary compounds will have higher turnover rates, through consumption and decomposition, than those producing more stable secondary compounds.

Keywords (max 6): *Cepaea hortensis*; decomposition; gastropods; herbivory; secondary compounds; snails
Introduction

Lichens are symbiotic associations in which a fungal partner (mycobiont) hosts cells of photobionts (green algae and/or cyanobacteria) that provide carbohydrates and, in the case of cyanobacteria, fix atmospheric nitrogen (N$_2$) (Nash 2008). Such symbiotic relationships shape plant-like life forms that play a prominent role in boreal forest ecosystems. Cyanobacterial N-fixing lichens may contribute significantly to the N cycling of those ecosystems in which they are dominant components (Nash 2008), such as boreal and temperate rainforests where species of Lobaria, Pseudocyphellaria and Sticta occur in abundance as epiphytes (Green et al. 1980; Antoine 2004). In addition, green-algal lichens are efficient accumulators of atmospheric nutrients from wet and dry deposition. For example, Knops et al. (1996) showed that the green-algal epiphyte Ramalina menziesii augments the input of total N, NO$_3$, organic N, Ca, Mg, Na and Cl in temperate deciduous forests. Further, lichen litter inputs may have significantly higher quantities of N and micronutrients than leaf litter from trees, because nutrients from tree leaves are usually resorbed back to the plant before abscission, while epiphytic lichen tissues fall to the ground with their nutrient concentration largely unchanged (Knops et al. 1991).

The turnover rates of carbon (C) and mineral nutrients entering the ecosystem from epiphytic lichens are determined by their tissue decomposition rates, and how quickly these tissues are consumed by invertebrates such as gastropods, springtails and mites. Both these processes are driven in part by functional characteristics of the thalli, including their concentrations of secondary compounds (Hättenschwiler & Vitousek 2000; Gauslaa 2005). Snails prefer specimens with artificially reduced levels of secondary compounds in both laboratory feeding experiments (e.g. Gauslaa 2005) and natural field conditions (Asplund & Gauslaa 2008). In addition, it is well know from studies on vascular plants that secondary compounds often greatly impair tissue...
decomposability and the release of nutrients during decomposition (Hättenschwiler & Vitousek 2000). However, to understand whether lichen secondary compounds have “afterlife” effects on lichen litter, we need to know the extent to which the lichens retain these compounds during senescence, and the consequences of this retention for breakdown of senesced lichen tissues through decomposition and consumption by invertebrates. In this light, while it is known that gastropods prefer senescent leaves to fresh leaves (Speiser 2001), it is unknown whether this is also the case with lichens.

In this study we explored how lichen palatability to snails changes during senescence for each of two contrasting epiphytic lichens (the cephalodial green-algal Lobaria pulmonaria and the cyanobacterial L. scrobiculata), and how these changes correspond to shifts in concentrations of secondary compounds and mineral nutrients. Lichenivorous invertebrates can play an important role in the breakdown of lichen litter (McCune & Daly 1994; De Oliveira et al. 2010) and the palatability of senesced lichens may, therefore, potentially affect their contribution to C and nutrient turnover in the ecosystem. As such, our study aims to improve knowledge about how senescence of lichens influences their palatability, and therefore to add to our understanding of how lichens contribute to forest ecosystem processes.

Materials and methods

Our study focuses on Lobaria pulmonaria and the closely related Lobaria scrobiculata. Both these N₂-fixing species are common in temperate and boreal rainforests but rather uncommon elsewhere. The main functional differences between them are the types of photobionts; L. scrobiculata has a cyanobacterial photobiont while L. pulmonaria has a green-algal photobiont and cyanobacteria in internal cephalodia. Both species exhibit the stictic acid chemosyndrome,
with the substances stictic, constictic, norstictic acids and other minor derivatives (Jørgensen 2007). In addition, L. scrobiculata produces m-scrobiculin and usnic acid (Jørgensen 2007). The invertebrate herbivore used in our study is the 14-22 mm wide Cepaea hortensis, which is a common and widespread broad generalist snail that climbs trees to feed on epiphytic lichens, including both of our studied lichen species (Asplund et al. 2010a).

Mature thalli of L. pulmonaria and L. scrobiculata were collected from four neighbouring Salix caprea trunks in an open Picea abies forest at Horka (64°26’N, 11°47 ‘E, 30 m a.s.l., Overhalla, Nord-Trøndelag, W Norway) in May 2011. The thalli were stored dry in the freezer until the start of the experiment; freezing is the recommended means of long-term storage of viable thalli for experimental studies (Honegger 2003). For each species, half of the collected thallus material was put in one 1.5 mm mesh litter bag of 30 × 30 cm and placed on the forest floor to engender tissue senescence. This involved placing these bags among Populus tremula litter in an old growth Picea abies forest at Kollåsen (59°45’N 10°57’E, 200 m.a.s.l., Ski, Akershus, SE Norway) on August 25 2011, where both species occur naturally. The other half of the thallus material was stored in a freezer until the start of the feeding experiment. The senesced lichens in the litter bags were brought into the lab after 6 weeks of undergoing senescence in the field, and left to dry at room temperature. Senesced thalli showed reduced maximal photosystem II efficiency when measured as described by Solhaug et al. (2003) using a portable, modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany). As such, mean ± SE values of FV/FM for senesced and living thalli, respectively, were 0.43±0.11 and 0.69±0.01 for L. pulmonaria, and 0.23±0.07 and 0.58±0.01 for L. scrobiculata. For the senesced lichens, photobionts of both species had turned more or less brown and the red-tinged mycobionts produced a red leachate when moistened. As such, we assume that both bionts were dying at this stage. For each species,
15 lobes of lichen thallus material from each of the two treatments (senesced thalli from litter
bags, and living thalli from freezer) were then randomly selected from the available material.
Each of these lobes was air dried and weighed (±0.1 mg). For each species, 15 plastic boxes
(each measuring 10 × 7 × 6 cm) were set up, and one senesced and one living lobe were placed in
each box; the lichens were sprayed with 3 ml water and 4 randomly selected snails were placed in
10 of the 15 boxes for each lichen species. These boxes were then closed with a perforated lid
and left for 24 hr at room temperature and natural day light but not in direct sunlight (as
described by Gauslaa 2005). The five boxes without snails were used to control for any non-snail
related changes in air-dry weight. After the 24 hr feeding experiment the lobes were left to air dry
and subsequently re-weighed. There were no significant differences in air-dry weight change
between the senesced and living thalli in the control boxes. Preference, sensu Lockwood (1998),
was calculated as the biomass consumed of one lobe divided by the pooled consumption of both
lobes in the box (hereafter referred to as feeding preference, expressed as a percentage). Thus,
when the snails consume equal amounts of each lobe the preference will be 50 % for both lobes.

For each species, ten lobes of senescent and of fresh material, each adjacent to the lobes selected
for use in the feeding experiment, were ground to powder in a ball mill. Approximately 20 mg of
the powder was extracted for four 30 min intervals in 2 ml acetone. The combined supernatants
were evaporated to dryness and 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 (Millipore, Billerica, Massachusetts,
USA) 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). In addition, the L. scrobiculata extracts were run through a
250 × 4.6 mm ODS Hypersil column (at 1 ml min⁻¹) to separate m-scrobiculin from usnic acid.
Compound identification was based on retention times, online UV spectra and co-chromatography of commercial standards. Nitrogen and phosphorus concentrations were determined by Kjeldahl digestion of a subsample of each lobe (n=5 for each species x treatment combination) followed by automatic colorimetric methods (Blakemore et al. 1987).

Results and Discussion

Senescence alters the palatability to snails of each of the two closely related lichen species but in contrasting directions (Fig 1). Senesced thalli of the cephalodial green-algal *L. pulmonaria* were more preferred than living thalli (Fig 1). The increasing palatability of *L. pulmonaria* thalli during senescence was concomitant with decreasing concentrations of secondary compounds such as the stictic acid chemosyndrome (Table 1), which has repeatedly been shown to deter snails (Gauslaa 2005; Asplund & Gauslaa 2008; Asplund 2011). In contrast, senesced thalli of the cyanobacterial *L. scrobiculata* were consumed less by the snails than were the living thalli (Fig 1). In addition to the stictic acid chemosyndrome, *L. scrobiculata* also produces usnic acid and *m*-scrobiculin that did not decline in concentration during the senescence process (Table 1). In this light, laboratory feeding experiments have shown that *m*-scrobiculin is a very effective lichenivore deterrent (Asplund et al. 2010b). Thus, even though the total concentration of secondary compounds was slightly reduced in *L. scrobiculata* during senescence, previous results suggest that the lichen should have remained sufficiently defended due to the unchanged concentrations of *m*-scrobiculin. However, concentrations of secondary compounds cannot explain why the senesced *L. scrobiculata* were avoided by the snails, and this avoidance could instead be due to the loss of easily utilized carbohydrates during the senescence process (Cooper & Carroll 1978; Dudley & Lechowicz 1987). As such, it has been suggested that invertebrate preferences for lichens are primarily based on their concentrations of easily digestible
carbohydrates rather than nutrients (Dubay et al. 2008). Further, L. scrobiculata may produce
cyanotoxins (Kaasalainen et al. 2012), which are quickly released from the cyanobacterial cells
during lysis (Watanabe et al. 2006). If these toxins remain in the thallus and are not leached out,
they may be consumed by lichenivores. However, globally only 12% of cyanobacteria associated
with lichens have the biosynthetic genes for producing cyanotoxins (Kaasalainen et al. 2012), and
it is therefore uncertain as to whether the lichens used in our study actually produce toxins.

We found higher concentrations of N and unchanged concentrations of P in senesced compared
to living thalli (Table 1). Since the entire lichen thallus falls to the ground and senesces, there is
no loss of mineral nutrients in tissues resulting from nutrient resorption, unlike the situation
frequently observed in vascular plants (Knops et al. 1991; Killingbeck 1996). Instead, N in
senescing thallus tissues appears to be stable, and initial mass loss during senescence is due to
loss of primary and secondary C-based compounds, resulting in increased N-concentrations at
least in the first few weeks. In an in situ decomposition study of Lobaria oregana, no significant
net N loss occurred until 17% of the initial mass was lost (Holub & Lajtha 2003). The high N
concentration of lichen litter compared with leaf litter suggests that lichen thalli may be an
important source of N in the ecosystem even when they have a lower total biomass relative to that
of leaves (reviewed by Nash 2008). Even though senesced plant leaves are low in N, they are
often favoured by gastropods (Speiser 2001) as a consequence of substantial reductions in
defence compounds during senescence (e.g. Newman et al. 1992). Thus, the palatability of
senesced versus living leaf material is driven more by secondary compounds than by nutrients.

Our results show compound-specific variation in the stability of lichen secondary compounds
during senescence, and that the change in palatability during senescence is species-specific.
These species differences could potentially have large effects on the rate at which C and mineral
nutrients in lichen tissues are released back into the ecosystem through invertebrate activity
(McCune & Daly 1994). In other words, lichens that produce secondary compounds, e.g. stictic
acid, which are quickly lost during senescence will have higher turnover rates (through both
consumption and decomposition) than those producing more stable secondary compounds. Litter
decomposition is a major driver of nutrient cycling in ecosystems, and key macrofaunal groups
such as gastropods play a fundamental role in this process (Swift et al. 1979; De Oliveira et al.
2010). Thus, understanding the controls of palatability of lichen tissue as it undergoes senescence
contributes to our knowledge of the role lichens play in affecting ecosystem processes.

Acknowledgement

This work was funded by a grant to JA from the Swedish Research Council (Vetenskapsrådet).
We thank Ulla Kaasalainen for helpful discussions.

References

Antoine ME, 2004. An ecophysiological approach to quantifying nitrogen fixation by Lobaria

Lichenologist 43: 491–494.

forest lichen Lobaria pulmonaria in broadleaved deciduous forests. Oecologia 155: 93–
99.


Table 1. Concentrations (mean ± SE) of secondary compounds (n=10), nitrogen and phosphorus (n=5) in living and senesced thalli of Lobaria pulmonaria and L. scrobiculata. P-values are from t-tests except where denoted with \(^a\) where a Wilcoxon rank sum test was performed because assumptions for parametric data analysis could not be satisfied.

<table>
<thead>
<tr>
<th></th>
<th>Lobaria pulmonaria</th>
<th>Lobaria scrobiculata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Living Senesced (P)</td>
<td>Living Senesced (P)</td>
</tr>
<tr>
<td>Stictic acid chemosyndrome (mg g(^{-1}))</td>
<td>39.0±2.0 20.4±3.9 &lt;0.001</td>
<td>31.1±2.2 19.4±3.5 &lt;0.05</td>
</tr>
<tr>
<td>m-Scrobiculin (mg g(^{-1}))</td>
<td>- - -</td>
<td>7.3±1.1 8.6±1.8 ns</td>
</tr>
<tr>
<td>Usnic acid (mg g(^{-1}))</td>
<td>- - -</td>
<td>6.0±0.5 7.0±1.8 ns</td>
</tr>
<tr>
<td>Total CBSCs (mg g(^{-1}))</td>
<td>39.0±2.0 20.4±3.9 &lt;0.001</td>
<td>44.4±2.0 35.0±4.6 &lt;0.05(^a)</td>
</tr>
<tr>
<td>Nitrogen (%)</td>
<td>2.1±0.08 2.4±0.08 &lt;0.05</td>
<td>2.6±0.07 3.0±0.03 &lt;0.01</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.16±0.03 0.15±0.02 ns</td>
<td>0.29±0.05 0.32±0.04 ns</td>
</tr>
</tbody>
</table>
Figure 1. Feeding preference (expressed as percentage of total consumption, mean ± SE) of the snail *Cepaea hortensis* when given the choice between living and senesced thalli of either *Lobaria pulmonaria* or *L. scrobiculata*. * and *** denotes p<0.05 and p<0.001, respectively. T-test was used for *L. pulmonaria* and the Wilcoxon rank sum test was used for *L. scrobiculata* because assumptions for parametric analysis could not be satisfied. Values in boxes represent total consumption (mean ± SE).