Chemical Variation Within and Between Individuals of the Lichenized Ascomycete *Tephromela atra*

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**Key Word Index**—Lichens; lichen compounds; herbivory; defense; chemical variation.

**Abstract**—HPLC-analysis was used to determine the concentrations of the lichen compounds alectoronic acid (depsidon), α-collatolic acid (depsidon) and atranorin (depsid) in the lichenized ascomycete *Tephromela atra* (syn. *Lecanora atra*) (Hudson) Hafellner from limestone walls on the Baltic island of Öland, Sweden. In 24 individuals of *T. atra* sampled on a stone wall, the pre-reproductive and reproductive tissue did not differ in the concentrations of alectoronic acid, collatolic acid and atranorin. The concentrations of the three lichen compounds were inter-correlated in the reproductive tissue, but not in the pre-reproductive tissue. Single individuals of *T. atra* ranged in area covered from 10.1 to 147.4 cm² (mean: 38.5 cm²; N = 24); 38.6% of this area was pre-reproductive tissue. However, the concentrations of the three lichen compounds were correlated neither with the total area covered by the lichen nor with the percentage of pre-reproductive tissue. This suggests that the concentrations of the lichen compounds do not change with increasing size (age) of the lichen. Analysis of specimens of *T. atra* from eight localities revealed a significant variation in lichen compounds (range between localities: alectoronic acid 0.60–3.26 μg/mg lichen dry weight (DW); collatolic acid 2.14–11.59 μg/mg lichen DW; atranorin 0.58–4.16 μg/mg lichen DW). The level of grazing observed in the lichens differed significantly among localities. However, no correlations between the concentrations of the three lichen compounds and the grazing damage to the lichens were found. Copyright © 1996 Published by Elsevier Science Ltd

**Introduction**

Lichens show a worldwide distribution, commonly growing on rocks and poorly developed soils such as those of arid lands and boreal–arctic regions, or as epiphytes on trees and shrubs (Crittenden and Porter, 1991). They may form the dominant floral elements in habitats that are characterized by extreme environmental conditions. Nearly 500 secondary lichen compounds have been identified so far (Culberson and Elix, 1989). These compounds are useful chemical characters in lichen taxonomy. However, little is known about their ecological role. The large concentrations of mostly phenolic secondary compounds that are accumulated in copious amounts (sometimes exceeding 20% of the dry weight) by many lichens have long been suspected to protect these symbiotic organisms from herbivores (Zukal, 1895; Stahl, 1904). Some lichen substances such as (−)-usnic acid or vulpinic acid demonstrated acute toxicity and feeding deterrenacy to larvae of the polyphagous insect *Spodoptera littoralis* (Noctuidae) (Emmerich et al., 1993). Other compounds such as oxyphysodic acid or fumarprotocetraric acid reduced larval growth of *S. littoralis* and caused malformations of imagines when added to the larval diet (Giez et al., 1994). On the other hand, there are specialized lichen feeders like oribatid mites (Seyd and Seaward, 1984), terrestrial gastropods

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(Baur et al., 1992; Hesbacher et al., 1995a) or Lepidoptera from the family Arctiidae (Rambold, 1985; Embacher, 1994; Hesbacher et al., 1995b) that are able to tolerate lichen compounds and even sequester them while feeding on the lichens.

Other roles attributed to lichen compounds include, for example, light screening effects. Cortical lichen substances commonly show variation in concentration along light gradients (Rundel, 1978). For example, populations of lichens with usnic acid (e.g. *Cladonia subveniens*) exposed to the sun are typically yellowish in colour, while populations living in a shaded environment are grey–green, indicating a lower concentration of usnic acid (Light screen function). Similarly, the concentration of the lichen compound parietin is positively correlated with light intensity (Hill and Woolhouse, 1966).

In the present study, we assessed the concentrations of secondary compounds in individuals of the lichenized ascomycete *Tephromela atra* from limestone walls in the grassland Great Alvar on the Baltic island of Öland, Sweden. A field study showed grazing damage in 33 (63.5%) of the 52 lichen species examined on limestone walls in this area (Baur et al., 1995). Among the lichen species considered, *T. atra* showed severe grazing damage (Baur et al., 1995). Potential lichen herbivores included different species of land snails and oribatid mites. Laboratory experiments demonstrated that individuals of the land snails *Baeoa perversa* and *Chondrina clienta* fed on *T. atra* (Fröberg et al., 1993; Baur et al., 1994). By eating lichens the snails can sequester secondary compounds such as atranorin (Hesbacher et al., 1995a).

Studies with higher plants have repeatedly shown that particular parts of the plants such as vulnerable young tissues and reproductive organs are better protected against herbivores by an accumulation of larger concentrations of secondary compounds than vegetative tissues (Siebertz et al., 1990; Hartmann and Witte, 1994). The aim of this study was to determine the concentrations of the lichen compounds alectoronic acid, collatolic acid and atranorin in the pre-reproductive and reproductive tissue of individuals of *T. atra* from a single locality. In particular, we examined (1) whether the concentrations of the lichen compounds differ between the two types of tissue, and (2) whether the concentrations of the lichen compounds are correlated with the size of the lichen and/or the proportion of reproductive tissue. We also assessed quantitatively the lichen compounds of *T. atra* from eight localities on Öland in order to examine (1) whether the concentrations of the three compounds differ between the localities, and (2) whether they are correlated with the grazing damage observed in the lichens.

![FIG. 1. CONCENTRATIONS OF ALECTORONIC ACID, COLLATOLIC ACID AND ATRANORIN IN PRE-REPRODUCTIVE (OPEN BARS), AND REPRODUCTIVE TISSUE (SOLID BARS) OF T. ATRA. Mean values ± SE of 24 individuals are given.](image-url)
Materials and Methods

Lichen samples. Specimens of *T. atra* were collected on the top of stone walls in the calcareous grassland Great Alvar on the Baltic island of Öland, Sweden (56°34'N, 16°27'E). To assess the within-individual variation in lichen compounds, we collected pre-reproductive (i.e. the outer zone of the individuals lacking fully developed apothecia), and reproductive parts (i.e. the central area of the individuals covered with fully developed apothecia) of *T. atra* individuals on a limestone wall 1.5 km SW of Ölands Skogsby on 7 August 1994 (8 individuals) and on 18 October 1994 (16 individuals). We determined the area covered by either type of lichen tissue in each lichen individual to the nearest mm² from drawings on transparent plastic sheets using a computer scanning programme. As an estimate of growth, we related the area covered by the pre-reproductive tissue of a lichen individual to its total area. For the biochemical analyses the lichen thalli were scraped with a knife from the horizontal surface of the stone wall.
To examine between-site variation in lichen compounds, we collected specimens of *T. atra* with a knife from the surface of eight stone walls in the Great Alvar in October 1993: (1) 0.3 km W of the southernmost tip of the marsh area Mokkelmossen on the northern side of the road connecting Resmo and Stenasa, (2) the church yard of Vickby, (3) 1.5 km SW of Oland's Skogshyt (the same locality as above), (4) 1 km E of Eriksöre, (5) 0.6 km E of Kalkstad, (6) at the southernmost edge of Skarpa Alby, (7) 0.1 km E of Tornör, and (8) Trestena fornminne, 2 km S of Södra Sandby. The stone walls were between 1.5 and 10 km apart from each other. At locality 2 the lichen material was obtained from the south-exposed, vertical wall of the church yard, at the other localities from the horizontal surface of stone walls. Thalli of six lichen individuals were collected at each locality. Individual lichens were between 0.2 m and 3 m apart from each other. Thalli that were growing in shaded positions were not collected. Grazing damage to each lichen was examined in the laboratory using a dissecting microscope. The level of grazing was classified as follows: no grazing = 0, low level of grazing = 1 (grazing damage visible but photobiont layer not exposed), moderate level of grazing = 2 (the photobiont layer was exposed in less than half of the lichen thalli), and high level of grazing = 3 (more than half of the lichen thalli with photobiont layer exposed; see Froberg et al., 1993).

**Quantification of lichen compounds.** Air-dried lichens were ground and extracted in a known amount of acetone at room temperature (20–22°C). The extracts were taken to dryness and stored in a freezer until HPLC-analysis.

**HPLC-analysis.** The dried extracts were dissolved in a known aliquot of methanol and directly subjected to HPLC-analysis. The HPLC system (Pharmlia) was equipped with a photodiode array detector (Waters). Samples were injected on a 5 μm Nucleosil 100 C 18 column (Knauer) 125 x 4 mm. Separation of the compounds took place by using a linear gradient from 100% A (50% MeOH, 50% H₂O adjusted to pH 2 with o-phosphoric acid) to 100% B (MeOH) in 15 min, followed by an isocratic segment for 5 min.

Identification of the lichen compounds was by comparison of retention times and by comparison of the online-recorded UV absorption spectra with those of commercially available lichen substances or with lichen compounds isolated and identified previously in the authors' laboratory. The lichen compounds were quantified by the external standard method. Concentrations of alectoronic acid, collatolic acid and atranorin (calculated as atranorin) are expressed as μg per mg lichen dry weight (DW).

**Results**

**Within-individual variation in lichen compounds**

HPLC-analysis revealed the presence of the lichen compounds alectoronic acid, collatolic acid and atranorin in all 24 specimens of *T. atra*. Alectoronic acid is reported for the first time for *T. atra*. Specimens collected in August differed neither in the concentrations of lichen compounds nor in their size (area covered) from specimens collected in October (t-test, *P* > 0.05 in all cases). Consequently, we pooled the data from the two sampling occasions for further analyses.

The concentrations of the three lichen compounds found in the pre-reproductive tissue did not differ from those determined in the reproductive tissue of *T. atra* (paired *t*-test; alectoronic acid: *t* = 0.27, d.f. = 23, *P* = 0.79; collatolic acid: *t* = 1.41, d.f. = 23, *P* = 0.17; atranorin: *t* = 0.79, d.f. = 23, *P* = 0.44). This indicates that the concentrations of the three lichen compounds are independent of type and age of tissue in *T. atra*.

In the reproductive tissue, the concentration of atranorin was correlated with those of alectoronic acid (*r* = 0.54, *n* = 24, *P* = 0.007) and collatolic acid (*r* = 0.51, *n* = 24, *P* = 0.010). In the pre-reproductive tissue, the three lichen compounds were not inter-correlated in the 24 lichen individuals (in all three cases, *P* > 0.08).

The 24 individuals of *T. atra* ranged in size from 10.11 to 147.44 cm² (mean cover of 38.52 cm²). The mean cover of the pre-reproductive tissue was 38.6% (range: 16.2–56.7%). However, the concentrations of the three lichen compounds were correlated neither with the total area covered by the lichen nor with the proportion of pre-reproductive tissue (Pearson correlation, in all cases *P* > 0.10). This suggests that the concentrations of the lichen compounds do not change with increasing size (age) of the lichen.

The percentage of pre-reproductive tissue was negatively correlated with the size of the lichen (*r* = −0.72, *n* = 24, *P* < 0.001). Thus, smaller lichens had a larger proportion of pre-reproductive tissue than larger ones. To examine whether individuals of *T. atra* that grow slower than the average of the population differ in
lichen compound concentration from those that grow faster, we calculated the regression lines of log-transformed lichen cover against the percentage of pre-reproductive tissue and analysed the residuals from the regression line in relation to the concentrations of the lichen substances. The concentration of atranorin in the pre-reproductive tissue was positively correlated with the residuals from the lichen cover—percentage of non-reproductive tissue relationship ($r = 0.44, n = 24, P = 0.03$). This indicates that individuals of *T. atra* with rapid growth contained a high concentration of atranorin in their pre-reproductive tissue. There were no correlations between alectoronic acid or collatolic acid and the residuals from the lichen cover—percentage of pre-reproductive tissue relationship (in both cases, $P > 0.1$).

**Between-site variation in lichen compounds**

HPLC-analysis of *T. atra* revealed the presence of the lichen compounds alectoronic acid, collatolic acid and atranorin at all eight localities (Table 1). The amount of alectoronic acid ranged from 0.60 to 3.26 $\mu$g/mg lichen DW at the eight localities (grand mean $= 2.36$ $\mu$g/mg). Corresponding values for collatolic acid were 2.14–11.59 $\mu$g/mg lichen DW (grand mean $= 7.95$ $\mu$g/mg) and for atranorin 0.58–4.16 $\mu$g/mg lichen DW (grand mean $= 1.86$ $\mu$g/mg). Thus, the mean concentrations of alectoronic acid and collatolic acid varied by a factor of 5 among the localities whereas the mean concentration of atranorin varied by a factor of 7. Specimens of *T. atra* from the eight localities differed significantly in the concentrations of the three lichen compounds (Table 1). Furthermore, the concentrations of the lichen compounds were intercorrelated (Pearson correlation; alectoronic acid–collatolic acid: $r = 0.68$, d.f. = 7, $P < 0.001$; alectoronic acid–atranorin: $r = 0.28$, d.f. = 7, $P = 0.06$; collatolic acid–atranorin: $r = 0.43$, d.f. = 7, $P < 0.01$).

All lichen specimens showed grazing damage. The level of grazing ranged from 0.5–3.0 at the eight localities (median of six lichen individuals at each localities; Table 1) and differed significantly among localities. However, there was no correlation between the concentrations of the three lichen compounds and the level of grazing (Spearman rank correlations; alectoronic acid: $r_s = 0.06$, d.f. = 7, $P = 0.66$; collatolic acid: $r_s = 0.14$, d.f. = 7, $P = 0.34$; atranorin: $r_s = 0.25$, d.f. = 7, $P = 0.09$).

**TABLE 1. VARIATION IN CONCENTRATION OF LICHEN COMPOUNDS (IN $\mu$g/mg LICHEN DW) AND GRAZING DAMAGE IN T. ATRA FROM EIGHT LOCALITIES ON ÖLAND, SWEDEN.** Mean values $\pm 1$ SE are given (median in grazing damage). $n = 6$ lichen individuals at each locality. Differences among localities were examined using Kruskal–Wallis tests.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Alectoronic acid $\mu$g/mg lichen DW</th>
<th>$\alpha$-Collatolic acid $\mu$g/mg lichen DW</th>
<th>Atranorin $\mu$g/mg lichen DW</th>
<th>Grazing damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.03 $\pm$ 0.67</td>
<td>7.27 $\pm$ 1.20</td>
<td>0.69 $\pm$ 0.30</td>
<td>1.5</td>
</tr>
<tr>
<td>2</td>
<td>2.44 $\pm$ 0.41</td>
<td>10.25 $\pm$ 1.19</td>
<td>1.24 $\pm$ 0.30</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>2.55 $\pm$ 0.73</td>
<td>8.58 $\pm$ 1.99</td>
<td>2.60 $\pm$ 0.93</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>3.26 $\pm$ 1.60</td>
<td>11.59 $\pm$ 3.08</td>
<td>2.08 $\pm$ 0.68</td>
<td>3.0</td>
</tr>
<tr>
<td>5</td>
<td>0.60 $\pm$ 0.06</td>
<td>2.14 $\pm$ 0.54</td>
<td>0.73 $\pm$ 0.29</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>2.62 $\pm$ 0.70</td>
<td>8.70 $\pm$ 1.34</td>
<td>1.90 $\pm$ 0.97</td>
<td>1.5</td>
</tr>
<tr>
<td>7</td>
<td>2.37 $\pm$ 0.89</td>
<td>6.89 $\pm$ 1.27</td>
<td>4.16 $\pm$ 0.77</td>
<td>1.0</td>
</tr>
<tr>
<td>8</td>
<td>2.02 $\pm$ 0.70</td>
<td>8.16 $\pm$ 1.43</td>
<td>1.61 $\pm$ 0.30</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Kruskal–Wallis:

$H' = 16.36$, $P = 0.022$

$\chi^2 = 17.78$, $P = 0.013$

$\chi^2 = 23.82$, $P = 0.001$

$\chi^2 = 17.27$, $P = 0.016$
Discussion

The present study provides evidence for between-site variation in the concentrations of alectoronic acid, collatolic acid and atranorin in *T. atra*. The differences in concentrations of lichen compounds may be explained by differences in abiotic conditions at the different localities. Herbivory by snails did not appear to influence the concentrations of lichen compounds, since we did not find any significant correlation between the level of grazing damage to the lichens and the lichen compound concentration.

Inducible chemical defense in lichens has so far not been studied. In higher plants, however, numerous examples for a stimulation of secondary metabolism as a consequence of herbivory exist. This phytochemical induction by herbivores may either result in *de novo* synthesis of allelochemicals such as proteinase inhibitors (Ryan, 1979, 1992) or in increased synthesis of constitutively expressed toxins such as the alkaloid nicotine in leaves of *Nicotiana sylvestris* that were damaged by herbivores (Baldwin, 1988).

It is possible that the secondary compounds present in *T. atra* are not involved in anti-herbivore defense, but serve other yet unknown roles for the lichens. This assumption is corroborated by laboratory experiments with larvae of the polyphagous noctuid *Spodoptera littoralis* which were not affected when fed on an artificial diet spiked with atranorin of natural concentrations (Giez et al., 1994), whereas other secondary lichen compounds such as (+)- and (−)-usnic acid or vulpinic acid caused severe larval mortality in the experiment (Emmerich et al., 1993).

In higher plants reproductive tissues are often protected by accumulation of large amounts of toxic or deterrent secondary compounds that protect these vulnerable plant parts from damage by herbivores. For example, flowering heads of *Ageratum houstonianum* (Asteraceae) are rich in insecticidal chromene derivatives (Siebertz et al., 1990), and flowering heads of *Senecio* spp. (Asteraceae) accumulate large amounts of toxic pyrrolizidine alkaloids (Hartmann and Witte, 1994). The observation that reproductive and pre-reproductive parts of *T. atra* did not differ in their concentrations of lichen compounds supports the assumption that these secondary compounds are probably not involved in anti-herbivore defense.

Atranorin is a cortical substance, and has been reported to occur in higher concentrations in old tissues of the lichen *Letharia vulpina* (Stephenson and Rundel, 1979). However, in this study no significant difference in atranorin concentration was found between young and old tissues of *T. atra*, nor was there any difference in atranorin concentration between large and small thalli. However, the atranorin concentration was correlated with growth. The biological role of atranorin is not known and cannot be deduced from this study. It is possible that atranorin (inactive) may actually serve as a storage product from which the plant slowly liberates, by a process of chemical degradation, another two bioactive compounds, β-orcinol methyl carboxylate (strongly antifungal) and atranol (potently antimicrobial) for defense from the attack by pathogenic microorganisms. Further investigations are required to elucidate the biological function of atranorin in *T. atra*.

The present study shows that fast-growing individuals of *T. atra* had a higher concentration of atranorin in the pre-reproductive tissue than slow growing individuals. This contradicts the growth rate hypothesis of Coley et al. (1985), which suggests that fast-growing plants should invest less in defense substances than slowly growing plants. However, the differences in growth observed in this study could be a result of small-scaled differences in microclimate (some individuals may occur at more favorable sites than others) or differences in the genetic composition of the lichen individuals.
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