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Short Communication

Difference in secondary compounds and chlorophylls between fibrils and main stems in the lichen *Usnea longissima* suggests different functional roles

Usnea is a species-rich and widespread lichenized fungal genus of well-lit parts of forest canopies (Motyka 1936, 1947; Clerc 1998). The bright greenish colour of these beard lichens reflects the presence of usnic acid in the cortex, which forms a thin, but dense sleeve around the trebouxioid photobiont in the outermost parts of the medulla. Usnic acid, a widely distributed dibenzofuran derivative produced by various mycobiont genera, strongly absorbs UV-B, but also the shortest PAR wavelengths (e.g. McEvoy *et al.* 2006, M. McEvoy, K. A. Solhaug and Y Gauslaa unpublished). Depending on species (Halonen *et al.* 1998), *Usnea* also contains a wide range of UV-B absorbing depsidones and depsides, though these are usually assumed to be confined to the medulla. Quantitative data on lichen compounds are rare in *Usnea* species, particularly with respect to the intrathalline variation.

This study deals with *Usnea longissima*, an epiphytic macrolichen often exceeding one metre in length. It belongs to the distinct subgenus, *Dolichousnea* (e.g. Wirtz *et al.* 2006), readily distinguished from other *Usnea* species by the long unbranched or scarcely branched main stems with a strongly disintegrating cortex. The main stems are corticate in the vicinity of their apices, though elsewhere the cortex flakes off, exposing the underlying white medulla. The main stems have short perpendicular side branches (fibrils), and on these the cortex remains intact. Most Norwegian material of *U. longissima* contains usnic and diffractaic acids, although one strain recorded by Thøgersen & Høiland (1976) had usnic and evernic acids. Elsewhere this species is chemically more diverse, as for example in North America (Halonen *et al.* 1998; usnic, evernic, diffractaic, barbatic and 4-*O*-demethylbarbatic acids) and Asia (Ohmura 2001; Mallavadhani *et al.* 2004: usnic, barbatic, 4-*O*-demethylbarbatic, squamatic acids and atranorin).

The main objective of this paper is to investigate possible quantitative differences in secondary chemistry within the thallus, and, more specifically, to search for chemical differences between fibrils and main stems. Since the cortex covers only the fibrils on mature thallus parts, we would expect the usnic acid to be restricted to these fibrils, whereas diffractaic acid should be more evenly distributed in the stems and branches(fibrils). Another additional objective is to quantify chlorophylls in fibrils and main stems, separately. An intrathalline covariation of usnic acid and photobiont cells, as quantified by chlorophyll measurements, would support the hypothesis that usnic acid protects the photobiont from solar radiation (e.g. Quilhot *et al.* 1991, 1996; Bjerke *et al.* 2005; Nybakken & Julkunen-Tiitto 2006). Finally, a compartmentalization of secondary compounds would suggest they had separate functions in the fibrils and main stems.

Usnea longissima Ach. was collected 18 May 2005 in steep, NE-facing low, old and open *Picea abies* stands in E Norway, Oppland, Toten (60°35′N, 11°02′E, 600–700 m a.s.l.). All the thalli studied had resulted from natural fragmentation, which is a common situation in natural populations (Gauslaa 1997), so the thalli lacked

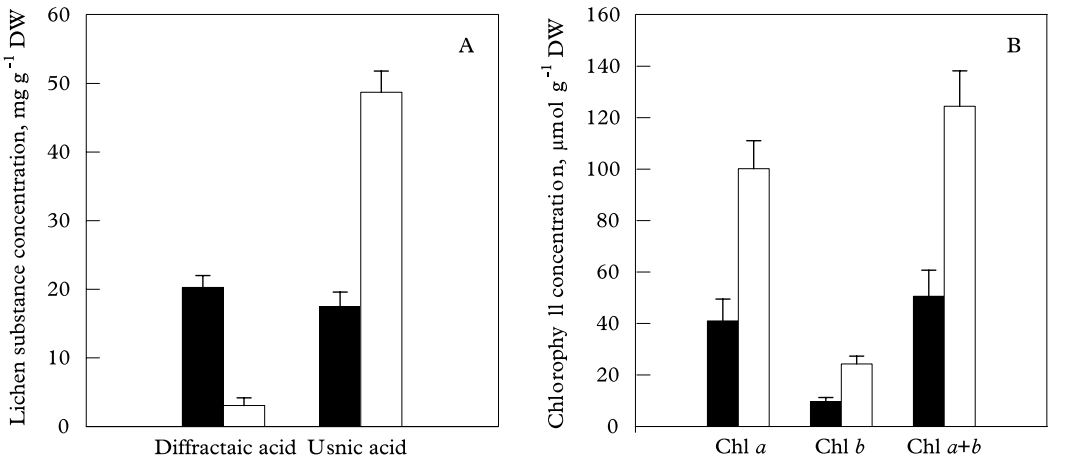

FIG. 1. Concentrations of lichen compounds and chlorophyll in main stems (black columns) and fibrils (open columns) of *Usnea longissima*. A, diffractic and usnic acids; B, chlorophylls *a* and *b*. Bars show 1 standard error (n=5).

stem tips with firm cortex. One piece of thallus was collected from each of five separate trees with rich populations, and the fibrils carefully removed from each thallus with scissors. To prevent damage to the main stem, tiny basal fragments of some fibrils were left behind. The air dry weight (d.wt) was measured of the fibrils (mean=107 mg) and the main stems (mean=35 mg).

For extraction of lichen substances each fraction of each air-dry thallus was submerged separately for 4 × 10 min in acetone in the laboratory. Preliminary experiments with additional grinding and re-extraction showed that acetone rinsing of intact thalli was sufficient for complete extraction of lichen compounds in this species (data not shown). No chlorophylls were extracted from air-dry and intact lichen pieces during acetone rinsing. The supernatants for the 4 extractions were combined and the extracts were left to stand in a fume cap until all the acetone had evaporated. After evaporation, the dry extract was re-dissolved in acetone (5 ml for main axis and 10 ml for fibrils) and analysed by HPLC according to the procedure of Nybakken & Julkunen-Tiitto (2006). The identification of lichen substances was based on retention times, online UV-spectra and co-chromatography of commercial standards of usnic acid (Sigma) and diffractaic acid (Gaia Chemicals). Quantification was done with standard curves of the two compounds.

Removal of lichen compounds prior to chlorophyll extraction is required in *U. longissima* to avoid chlorophyll degradation during chlorophyll extraction. After acetone-rinsing, the lichen fractions were hydrated and then extracted in 5 ml N,N-dimethylformamide for chlorophyll analyses. All samples were kept dark at 4°C for 4 days to allow a complete extraction of chlorophylls before the absorbance was measured at 647, 664, and 750 nm, and concentration (µmol g^{-1} d.wt) of chlorophylls *a* and *b* were calculated (Porra *et al.* 1989).

The fibrils comprised approximately 75% of the total thallus dry weight. The average total concentration of secondary compounds (usnic and diffractaic acid) in a thallus comprising both fibrils and main axis was 4·8% in dry matter. Usnic acid occurred in the highest concentration (4·1%). Main stems and fibrils differed substantially in the concentration of secondary compounds (Fig. 1A). Most diffractaic acid was located in the main stem, with a concentration approximately seven times higher than in fibrils. In contrast, the usnic acid concentration was nearly three times higher in the fibrils than in the main axis. Chlorophyll concentration was also highest in the fibrils (Fig. 1B) with approximately 90% of total chlorophyll and usnic acid present. Here it should be noted that even this is an underestimation, as the main stems bore the extreme basal portions of the fibrils. The ratio of usnic acid to chlorophyll was the same in both the main stems and the fibrils, but this was not the case with diffractaic acid.

Such intrathalline variation in chemistry suggests different functional roles of usnic and diffractaic acids. Usnic acid may protect the fungal and/or algal partner in a lichen thallus from excess solar radiation. Buffoni-Hall *et al.* (2003) reported lichen DNA-damage, indicating possible myco-

biont damage due to UV-B exposure. However, the strong co-variation in chlorophylls and usnic acid is consistent with a photoprotective role of the cortical usnic acid on symbiotic green algae (e.g. Quilhot *et al.* 1991; Nybakken & Julkunen-Tiitto 2006; McEvoy *et al.* 2006, 2007).

Usnea longissima is unique in the sense that it is one of few epiphytic lichens that lacks a permanent holdfast. It is a vagant lichen (Gauslaa 1997) and adheres to the supporting canopy branch by wrapping around it. Photosynthesis takes place mainly in the fibrils, whereas the main stem provides structural support, and/or an internal store of fixed carbon. However, the contact zones between the main thallus stem and the tree branch easily become infected or damaged. This weakens the structural support of the thallus, and so leads to frequent fragmentation (Gauslaa 1997). Thereby, the main stem also facilitates dispersal. Some secondary lichen compounds may deter various micro-organisms (Lawrey 1986; Candan *et al.* 2006), including parasitic fungi (Lawrey 2000; Halama & Van Haluwin 2004), as well as small grazing herbivores (Lawrey 1983; Gauslaa 2005). Diffractaic acid may play a defence role against such biotic agents in the main stem of *U. longissima*.

In conclusion, fibrils in *U. longissima* are the photosynthetic organs, whereas main stems have other functions. The strong co-variation in chlorophylls and usnic acid is consistent with a photoprotective role of the usnic acid.

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