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BRIEF COMMUNICATION

Changes in spectral reflectance of a foliar lichen *Umbilicaria hirsuta* during desiccation

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Abstract

Water potential (ψ_w) and water saturation deficit (WSD), and several reflectance (R) indexes were assessed in an aerophytic lichen *Umbilicaria hirsuta* (Sw. ex Westr.) Hoffm. The water index (WI, R_{900}/R_{970}) and normalized difference vegetation index, NDVI [$(R_{900}-R_{680})/(R_{900}+R_{680})$] were strongly correlated both with the ψ_w and the WSD of lichen thalli. No significant changes during desiccation were found in structural independent pigment index, SIPI [$(R_{800}-R_{445})/(R_{800}-R_{680})$]. Sensitivity of the spectral detection of water status was rather small at high hydration level (WSD < 25 %, or ψ_w > -1 MPa), but this is not much limiting its value and potential use, because physiological processes in lichens are usually inhibited at much lower values of ψ_w than in leaves of vascular plants.

Additional key words: reflectance indexes, upper cortex, water deficit, water potential.

Physiological processes in poikilohydrous lichens are closely dependent on water status in their thalli, and this is again immediately influenced by external water availability and conditions for evaporation. The intrathalline water status can be expressed in terms of water content, water deficit, or water potential. Although it is not much difficult to assess these characteristics in laboratory conditions, it is nearly impossible to use the standard physiological methods (*e.g.*, gravimetric or hygrometric) in field studies with lichens firmly attached to stones or to other substrates. Conductometric or impedance methods were developed and applied in some studies (Coxson 1991, Schuster *et al*. 2002), but they are not suitable for sufficiently precise and non-destructive detection of heterogeneity in water status over lichen thalli.

As a more promising approach for such studies seems to be remote sensing and analysis of diffuse spectral reflectance, which has already been used for detection of water status and some other physiologically important characteristics in plant leaves (for review see Peñuelas and Filella 1998). This approach is based on changes in spectral characteristics of radiation reflected from internal structures of a leaf, not only from its surface, as in the case of specular reflectance. Radiation penetrating into a leaf is scattered and diffused at interfaces of leaf components with different refractive index (wet cell walls, air-filled intercellular spaces, cytoplasm, organelles), and selectively absorbed by chlorophylls, carotenoids, and some other molecules (Wooley 1971, Gausman 1977, Peñuelas and Filella 1998).

Water is also a selective absorber of radiation, mainly in the infrared region. The strongest absorption properties of water molecules were found at longer wavelengths (middle-infrared, namely at 1450, 1940 and 2950 nm, Danson *et al*. 1992, Peñuelas *et al*. 1993). Unfortunately, this region is not much practical for assessment of water status in plants, because of rather shallow penetration of long-wave radiation into the plant structures and of some noise caused by changes in air humidity above samples (Peñuelas *et al*. 1993, Peñuelas and Inoue 1999). In addition to this, the easily portable narrow-band width spectroradiometers are seldom equipped with a detector sufficiently sensitive beyond 1100 nm.

Reflectance changes in the near infrared radiation region (NIR, 700 - 1300 nm) can also be used for

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Abbreviations: NDVI - normalized difference vegetation index; NIR - near infrared radiation; R - reflectance; SIPI - structural independent pigment index; WI - water index; WSD - water saturation deficit; ψ_w - water potential.
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detection of water in biological samples, because one of the water absorption bands is situated at 950 - 970 nm. NIR penetrates more deeply into the measured structures than middle-infrared, and so the reflectance depends more on the water content of the whole sample than on water located in the uppermost layers. The applicability of NIR for detection of leaf water status was explored in details by Peñuelas *et al*. (1993, 1996, 1997). They suggested as the most advantageous to use for such detection the ratio between the reflectance at 900 nm (reference) and reflectance at 970 nm (most influenced by absorption in water). The ratio (R_{900}/R_{970}) is denoted as "water index" (WI, Peñuelas *et al*. 1997).

Reflectance in the near infrared region is unfortunately very sensitive not only to the presence of water, but also to the shape and orientation of mesophyll cells, which may vary substantially at decreasing pressure potential (Bowman 1989, Danson *et al*. 1992, Wooley 1971). As shown by Peñuelas and Inoue (1999) and Wooley (1971), the structural changes caused by progressive desiccation are species-specific. Water deficiency may cause also some changes in the ratio between carotenoids and chlorophylls (mainly due to partial chlorophyll degradation), which results in specific changes of reflectance in visible region. These changes may be followed by several indices, as, *e.g.*, by normalized difference vegetation index, NDVI [$(R_{900}-R_{680})/(R_{900}+R_{680})$], or structural independent pigment index, SIPI [$(R_{800}-R_{445})/(R_{800}-R_{680})$]. As demonstrated by Peñuelas *et al*. (1999), relative water content in leaves may, therefore, be better correlated with the ratio WI/NDVI than with the WI itself.

In contrast to many reflectometric studies done with leaves of vascular plants, only a very limited amount of experimental work has been done to characterize diffuse spectral reflectance of lichens and utilize the results in lichen physiology. It has still been questionable if the same approach elaborated for reflectometric analysis of leaves could be used in studies of lichens because of their structural peculiarities. The upper surface of a lichen thallus is not covered by a transparent cuticle and epidermis, but by a cortex, formed by dense aggregation of fungal hyphae fused together by polysaccharidic substances. In aerophytic lichens of the family *Umbilicariaceae* the upper cortex is comparatively thick structure (> 40 μm, Valladares 1994), and usually strongly reflexive when not fully saturated with water. This may represent a serious obstruction in utilization of reflectometric method to indication of structural and functional changes in lichens. The aim of the present preliminary study was to assess applicability of spectral reflectance analysis originally derived for remote sensing of water status in leaves of vascular plants to similar studies with lichens.

The experiments were done with young, more or less homogeneous thalli of foliose lichen *Umbilicaria hirsuta* (Sw. ex Westr.) Hoffm., freshly collected in their natural habitat (perpendicular granitic rock walls in the Oslava river valley near Brno, Czech Republic). The measurements started with samples fully saturated with water (after 12 h of exposure on wet filter paper in a Petri dish), but without any visible liquid water on external surfaces. The samples were mounted on special supporting plates made from stainless wire net and placed on analytical balances for continuous recording of mass changes during desiccation. Measurements of spectral reflectance and water potential of the samples (for more details on these techniques see below) were taken about five to ten times during each desiccation cycle (from full water saturation to nearly total water loss at ambient conditions). During each measurement, a quick sequence of 10 reflectance scans covering the area of the tested thallus was taken and averaged. The external conditions were nearly stable - air temperature was kept in the course of experiments at 20 ± 1 °C, and relative air humidity at 50 ± 5 %. Continuous irradiance of the samples was 200 ± 50 μmol m^{-2} s^{-1} of photosynthetically active radiation. The external variables were recorded during experiments in 1-min intervals using a miniature data logger *HOBO* (*OnSet Computers*, Bourne, USA). In addition to this basic procedure giving information on reflectance characteristics at different water potential, water saturation deficit (after determination of dry mass of samples) was calculated.

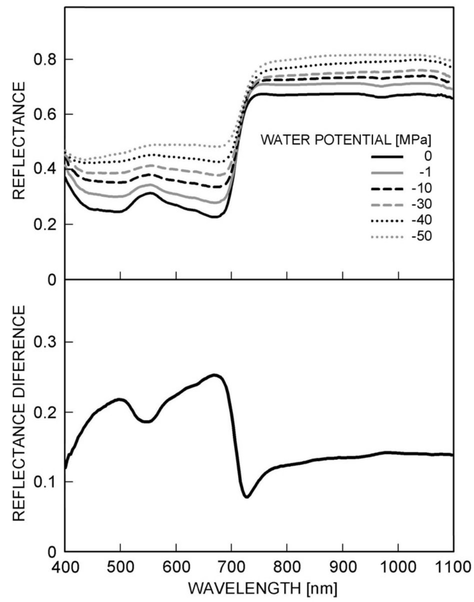

Fig. 1. Changes in spectral reflectance of *Umbilicaria hirsuta* thalli during progressive desiccation from full water saturation (water potential = 0 MPa) to severe dehydration. The difference between the highest and lowest reflectance curves (taken at 0 and -50 MPa, respectively) is depicted in the lower graph.

Spectral reflectance measurements were done with a reflectometer *UNISPEC* (*PP Systems*, Haverhill, USA) equipped with NIR enhanced detector (300 - 1100 nm), internal light source (halogen lamp), and bifurcated foreoptics with a special clip for stabilisation of the foreoptics orientation at a 60° angle to the sample plane. To calculate reflectance, spectral radiance scans of the

samples was divided by radiance scans of a 99 % reflective standard (*Spectralon*, *Labsphere*, North Sutton, USA). *UniWin* software was used for analysis of reflectance spectra and for calculation of spectral indices. In most cases, the following reflectance indices were derived from spectral reflectance curves (R denotes reflectance and the subscripts refer to specific spectral wavelength): water index, $WI = R_{900}/R_{970}$,
normalized difference vegetation index,
$NDVI = (R_{900}-R_{680}) / (R_{900}+R_{680})$.
structural independent pigment index,
$SIPI = (R_{800}-R_{445}) / (R_{800}-R_{680})$,

Water potential measurements were done using a *Dew Point Hygrometer HR-33T* (*Wescor*, Logan, USA) with a custom made set of equilibration sample chambers. Water saturation deficit (WSD) of all samples was calculated from actual fresh mass (FM_A), fresh mass at maximum water saturation (12 h on wet filter paper) (FM_S), and from dry mass (DM) of the sample:
$WSD [\%] = [(FM_A - DM)/(FM_S - DM)] \times 100$.
Analytical balances with *RS-232* output to a notebook were used for continuous monitoring of sample fresh mass during desiccation experiments.

Reflectance of the measured lichen thalli gradually increased with decreasing water potential at all wavelengths (Fig. 1). The increase was more pronounced in the visible part of spectrum (by about 100 % of the value of wet samples), than in the near infrared region (up to 20 %). It should be noted, that due to relatively high amount of extracellular water in wet samples, physiologically important decrease of water potential (below -1 MPa) was recorded at rather high values of

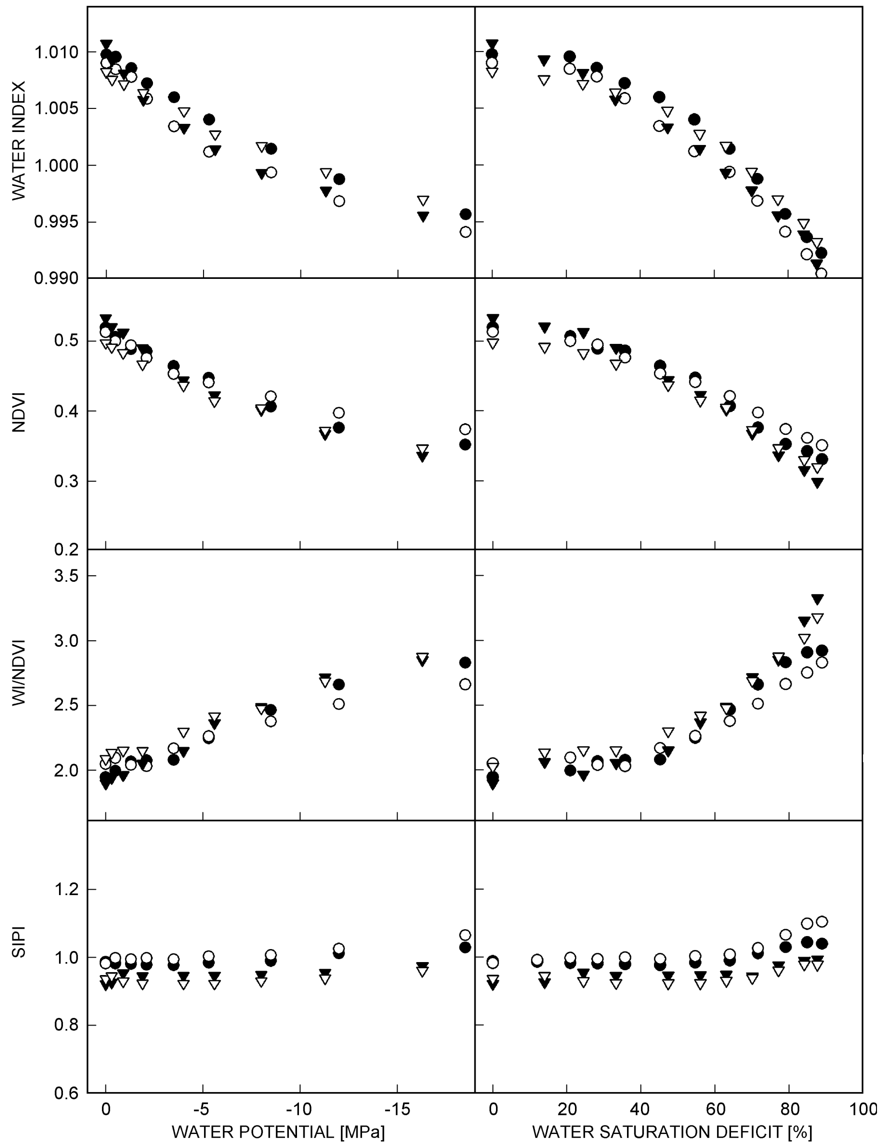

Fig. 2. Dependence of several reflectance indices on intrathalliner water potential (left column), and on water saturation deficit (right column) in desiccating thalli of *Umbilicaria hirsuta*. Data were calculated from multispot and averaged measurements on four thalli. Different symbols denote each of the measured thalli.

water loss, above about 25 % of WSD. The changes of reflectance parameters were followed in all experiments until the samples attained water saturation deficit about 95 %, which was equivalent to dry state at the given ambient temperature and air humidity. The values of water index (WI) derived from spectral reflectance measurements were strongly correlated ($r > 0.95$) both with the water potential and the WSD of lichen thalli.

Surprisingly, similar non-linear correlation was found also between reflectance index NDVI and both characteristic of intrathalliner water status. NDVI is usually considered as an indicator of chlorophyll content in photosynthesizing organs and chlorophyll degradation under stress conditions. The causes and indicative value of the observed changes in NDVI during desiccation of our lichen samples were most probably more complex than in the case of leaves of homoiohydric plants (*cf.* Peñuelas and Inoue 1999), because chlorophyll pigments in algal lichen symbionts are more stable than pigments in leaves at severe water deficiency (Kappen and Valladares 1999). On the other hand, the reflectance properties of lichen upper cortex could interfere. Relatively slower increase of reflectance in the NIR region during desiccation (in comparison to more increasing reflectance in shorter wavelengths) would result in decreasing values of NDVI even at the same intact chlorophyll content in the lichen samples. In contrast to finding of Peñuelas and Inoue (1999), rationing of WI by NDVI not improved the indication value which possess both components alone.

The index SIPI (expressing the ratio between carotenoids and chlorophylls) was reported by Peñuelas and Inoue (1999) as another suitable indicator of water deficiency in leaves of vascular plants. The carotenoids are usually more stable than chlorophylls during desiccation, which is expressed in increased values of SIPI in water stressed leaves. The index SIPI, unlike the NDVI, is nearly independent on structural changes (others than pigment content) in the measured sample. No significant change in SIPI was found in our tested lichen samples during desiccation, which may be considered as an indirect indicator of chlorophyll stability in symbiotic algae. Consequently, it supports our hypothesis, that the observed changes of NDVI in desiccated lichen thalli were not caused by chlorophyll degradation, but more likely by structural changes in the upper cortex.

It is possible to conclude, that the detection of water status in thalli of foliar lichen *Umbilicaria hirsuta* using spectral reflectance analysis is principally possible within broad range of water deficiency. Reflectance signal in the near infrared region (at 970 nm) has been found as the most reliable water status indicator for the tested lichen specimens. Sensitivity of the spectral detection of water status was rather small at high hydration level (WSD < 25 %, or water potential > -1 MPa), but this is not much limiting its value and potential use, because physiological processes in lichens are usually inhibited at much lower values of water potential than in leaves of vascular plants (Barták *et al*. 2000, 2005, Nash *et al*. 1990, Kappen and Valladares 1999).

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