Light might regulate divergently depside and depsidone accumulation in the lichen *Parmotrema hypotropum* by affecting thallus temperature and water potential

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Abstract: Depsides and depsidones are the most common secondary products uniquely produced in lichens by the fungal symbiont, and they accumulate on the outer surface of its hyphae. Their biological roles are subject to debate. Quantitatively the compounds typical of a given lichen can vary dramatically from thallus to thallus. Several studies have addressed whether this variability is correlated with the light reaching different thalli, but the conclusions are contradictory. We addressed the question with the lichen *Parmotrema hypotropum* growing on unshaded, vertical tree trunks, a controlled natural environment where the light absorbed by each thallus over its lifetime is the only major position-dependent variable. The exact north-east-south-west orientation of each thallus was used to calculate its yearly light exposure based on astronomical and meteorological considerations. The calculated irradiation around the trunk, distributed over a continuous 40-fold intensity range, then was compared with the amount of compound per unit thallus weight, determined by quantitative thin layer chromatography. *P. hypotropum* accumulates the depside atranorin in the cortex and the depsidone norstictic acid in the medulla and around the algae. A direct correlation was observed between the yearly amount of light reaching the lichen and the amount of atranorin. In contrast, the amount of norstictic acid decreased with increasing light. Although we did not measure thallus temperature and water potential, a unifying interpretation of these and other published data is that depside/depsidone accumulation in lichens is mediated by localized changes in temperature and water potential produced by light absorption within each thallus. This suggests water relations-based functions for depsides and depsidones.

Key words: lichen compounds, polyketides, solar radiation, symbiosis, thermal stress, water relations

INTRODUCTION

Lichen fungi produce a wealth of secondary compounds, many found also in nonlichen fungi, but are best known for their unique polyketides, the depsides, depsidones and dibenzofurans that typically encrust the hyphal and algal surfaces of lichens. Lichen-specific compounds are acidic and water repellent. The core chemistry of this acetyl polymalonyl-derived family of about 600 compounds is uniform across thousands of lichen species and generally consists of two phenolic rings with various substituents, joined by ester and/or ether linkages (For reviews see Fahselt 1994, Elix 1996, Huneck 1999). The occurrence of specific sets of compounds in lichens can be useful in defining their taxonomy (For a review see Lumbsch 1998). For instance *Parmotrema hypotropum* (Nyl.) Hale, the lichen in this study, is characterized by the co-occurrence of the depside atranorin and the depsidone norstictic acid (Hale 1965, 1974).

Although a taxonomic group may have characteristic compounds present in all its thalli, the amount of individual compounds can vary greatly (Stephenson and Rundel 1979, Fahselt 1984, Bjerke et al 2002). Several studies have investigated this variability in relation to UV and visible light exposure. The emphasis on light is due to the fact that some lichen phenolics are pigmented and most absorb in the UV range, suggesting possible photoprotective functions for these compounds. This possibility has fairly consistent support (Solhaug and Gauslaa 1996, Nybakken et al 2004 and references therein) for melanins and for anthraquinone pigments such as parietin, which occur in the upper cortex of some lichens but are not unique to them. However, for the lichen-specific depsides, depsidones and dibenzofurans, the connection to visible or UV light is not clear and neither are their functions in the symbiosis. The amounts of usnic acid, a widely distributed cortical dibenzofuran derivative, are positively correlated to...
MATERIALS AND METHODS

Lichen collection and weighing.—To rule out interferences from shading that would spuriously affect the results, well isolated trees with cylindrical and vertical trunks were chosen. Two red maple trees were found in open areas. A sampling area covering the trunk circumference was selected that showed a uniform lichen distribution and no significant shading from the crown. Eighty-three Parmotrema hypotropum thalli, found within an 8 cm wide band running horizontally about 1 m above the ground, were harvested from the trunk. These included all but the smallest thallus initials. A tape measure was fastened around the trunk above the area chosen for sampling, running north, east, south, west, north with the zero on north as determined with a compass. The position of each thallus relative to the zero on the tape was recorded with a plumb string from tape to thallus center. Each thallus was removed and placed in individual tubes. Before weighing, thalli were equilibrated 24 h at room humidity (~ 40%), and extraneous material was removed carefully with tweezers. Weights were 0.7–28.36 mg. Thalli were weighed also after compound extraction (see below). In tabulating the data, tape distances were converted to degrees and corrected for magnetic vs. true north displacement in this area (36.02N, 78.95W) at the time of data collection. The local value for the magnetic declination (8°32’) was obtained through the National Geophysical Data Center (http://www.ngdc.noaa.gov/seg/geomag/jsp/struts/calcDeclination).

Calculation of the integrated yearly light intensity distribution around a vertical cylinder.—To describe the distribution of the yearly solar radiation reaching a vertical, exposed cylindrical trunk at the latitude of Durham, North Carolina, (36.02N) we needed to know the amount of sunlight reaching the ground from every position in the sky over the year. A sun chart (Fig. 1) provided the sun’s positions vs. time, while the readings from a Raleigh-Durham weather station provided the values for incident radiation vs. time. These two datasets were combined as follows.

The sun chart represents the sky’s hemisphere as a cylindrical projection subdivided into 432 square “sky units” (Fig. 1). The dates of the sun’s trajectories are marked. The position of the sun at each hour is indicated by the lines crossing the trajectories. The length of the trajectory between two hour-lines was taken to represent 100% of the light emanating from that region of space during that hour on that date. The fractional length of the hourly trajectory within each sky unit was used as a measure of the percent light emitted from that unit (Fig. 1 inset).

Values for ground-level radiation were derived from hourly measurements by the Raleigh-Durham station of the National Weather Service. The station’s data are stored in the hourly data files (http://rredc.nrel.gov/solar/old_data/nsrdb/hourly/) of the National Solar Radiation Database (NSRDB). From this database the values for direct normal radiation (DNR) were selected as indicators of the radiation reaching the ground per hour. DNR is defined as the amount of solar radiation received per hour within a 5.7’ field of view centered on the sun. Its hourly values are listed in the database as energy (in watt-hours)/m². To have a
good average representation of ground-level radiation around the dates of the sun chart trajectories, the radiation values from 4 d before to 4 d after the trajectory dates were averaged for 1988, 1989 and 1990, the last years for which complete data are available. (TABLE I represents the data compilation and averages for the month of January as an example. Each trajectory is labeled with its corresponding month and day. Each hour is represented by a line cutting through the trajectories. The x axis represents the 360° of the azimuth running north, east, south, west, north and is subdivided into 24 sections; each section defines a vertical column representing a 15° wide “sky sector” (the 150°–165° sky sector is shaded as an example). The y axis represents the sun’s elevation 0°–90° (θ angle; see text) and is subdivided into 18 sections, each defining a 5° wide row. The chart thus comprises 432 sky units. The inset enlarges the Jan 17 trajectory at 1–2 PM and provides an example of how the average incident radiation derived from weather station measurements was partitioned graphically between the different sky units in the sun chart; of the 384.33 Wh/m² average radiation reaching the ground Jan 17 at 1–2 PM (TABLE I), 33%, 47% and 20% were assigned respectively to the top left, top right and bottom right sky units in the inset. (See text for detail).

DNR data are obtained through a moving detector surface always centered on the sun and perpendicular to the sun-detector axis. However, only the horizontal component of this radiation is absorbed by the vertical surface of the trunk. For a given sun elevation, the horizontal component equals the DNR value times cos θ, where θ is the sun elevation. Therefore, to obtain the energy reaching the trunk surface from each sky unit in the sun chart, each hourly DNR average was multiplied (i) by the fractional length of the corresponding trajectory in that sky unit (FIG. 1 inset) and (ii) by cos θ (FIG. 1, TABLE I). Finally the horizontal radiation values coming from all sky units in a sector (see FIG. 1) were added together to obtain the cumulative yearly radiation reaching the trunk from a particular NWES direction in the Durham area (thick line in FIG. 2A). To obtain the distribution of the horizontal radiation around the trunk, the radiation from each sky sector was multiplied by the sine of its incidence angle onto the trunk’s surface. The resulting distributions (FIG. 2A dashed lines) then were integrated to generate the final yearly radiation distribution on the trunk (FIG. 2B).

FIG. 1. Sun chart for the Durham, N.C., area. The diagram, obtained from the University of Oregon Solar Radiation Monitoring Laboratory (http://solardat.uoregon.edu/SunChartProgram.html), is a cylindrical projection of the sun’s trajectories at Durham’s latitude. For clarity only Dec–Jun trajectories are shown. Jul–Nov trajectories cover the same area in a similar way. Each trajectory is labeled with its corresponding month and day. Each hour is represented by a line cutting through the trajectories. The x axis represents the 360° of the azimuth running north, east, south, west, north and is subdivided into 24 sections; each section defines a vertical column representing a 15° wide “sky sector” (the 150°–165° sky sector is shaded as an example). The y axis represents the sun’s elevation 0°–90° (θ angle; see text) and is subdivided into 18 sections, each defining a 5° wide row. The chart thus comprises 432 sky units. The inset enlarges the Jan 17 trajectory at 1–2 PM and provides an example of how the average incident radiation derived from weather station measurements was partitioned graphically between the different sky units in the sun chart; of the 384.33 Wh/m² average radiation reaching the ground Jan 17 at 1–2 PM (TABLE I), 33%, 47% and 20% were assigned respectively to the top left, top right and bottom right sky units in the inset. (See text for detail).
**Table I. Compilation matrix for the data from the National Solar Radiation Database**

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Only the January data are shown as an example. The tabulated values are in watt-hours/m² and represent direct normal radiation (see text for the definition) measured by the Raleigh/Durham station of the U.S. National Weather Service. The measurement dates and hours are listed respectively in the first column and the first row. The values represent the radiation measured during the hour preceding the indicated times. The dates, from 1988, 1989 and 1990, comprise Jan 17 (the date of the trajectory in the sun chart, in bold) and the four days preceding and following it. The averages (last row) were taken to be proportional to the radiation reaching the ground in January. (The sun angles θ (second row, in degrees; b.h., below horizon) were derived from Fig. 1.)
Scanning electron microscopy.—Thallus cross sections produced manually with a razor blade were placed on the scanning microscope stub with a sectioned face upward, coated with gold and palladium and scanned with an FEI Philips XL30 ESEM.

Compound extraction.—Compounds were extracted from complete thalli that weighed less than 5 mg. For larger thalli, 5 mg fragments were dissected, weighed and used for extraction. Each thallus or thallus fragment was placed in 1 mL acetone in a tightly capped 50 mL polypropylene tube. Tubes were placed in racks and shaken at 200 rpm at room temperature 30 min. Taking care to avoid losses, each extract was transferred to a graduated 15 mL polypropylene tube with a dedicated Pasteur pipette and the lichens in the original tubes were extracted again as before, but for 60 min. The second extract was pooled with the first, the extraction was repeated a third time 60 min, and the last extract was pooled with the previous two. Pilot experiments showed that this extraction protocol removed 95% of the compounds from Parmotrema (not shown), an extraction response similar to that of other lichens (Solhaug and Gauslaa 2001). Depending on the dilution needed for each extract (see next section) volumes were adjusted by adding acetone or by evaporating it. When reducing volumes, care was taken to keep samples damp because sometimes compounds do not fully redissolve after drying. Tubes were stored at 4 C. Chromatography was performed within a few days of extraction.

Compound quantification.—Atranorin and norstictic acid were chromatographed on TLC plates (Silica Gel 60 F254, EM Science) in solvent B (Culberson et al. 1981). Spots were quantified with a method adapted from BeGora and Fahselt (2000). TLC plates were photographed immediately after evaporation of the solvent by placing each in a dark enclosure equipped with a digital CCD camera (Cohu) and illuminated with a shortwave UV lamp. The digital images were processed with NIH Image (version 1.62 f) by subtracting the plate background and scanning each lane. Peak areas, representing spot intensities, were recorded and processed with Microsoft Excel. All spot intensities were normalized to an extracted thallus weight unit of 24 mg. Here the term “concentration” refers to compound amount per 24 mg.

To be accurately quantified, compounds were loaded onto the plates within the linear proportionality range between amount of compound per spot and spot intensity (pixels). This range was determined in preliminary TLC runs. Convenient working extract volumes for this lichen were 0.5 mL acetone/mg of extracted thallus (with 12 mL TLC loading volume) or 1 mL acetone/mg of extracted thallus (with 6 mL TLC loading volume). Each sample was chromatographed at both these dilutions and loading volumes to cover the variability range of norstictic acid and atranorin.

RESULTS

Light distribution around an unshaded tree trunk.—More radiation reaches the trunk from the east than from the west (Fig. 2A, thick line). This is because the yearly average cloudiness and moisture are higher in

![Fig. 2](image-url)

**Fig. 2.** Outline of the final steps in determining the yearly light distribution on the surface of a vertical tree trunk. In both panels the ordinate represents energy and the abscissa the azimuth of 0–360° in the N-E-S-W direction. **Panel A:** The thick line represents the ground-level distribution of the horizontal component of yearly sunlight at our latitude. Each large dot corresponds to the cumulative energy from the sky sector at that position, calculated as described above (Fig. 1). The horizontal radiation distributes itself around the trunk surface as a function of the sine of the angles of incidence onto the surface, as indicated by the dashed curves departing symmetrically from each large dot. **Panel B:** distribution of light around the trunk surface, obtained through summation of all the values of the dashed lines in panel A. Because the energy averages (Table I) refer to 1 d per mo, the summation in panel B corresponds to about 1/30 of the wh/m² hitting the tree surface during the year.
the afternoon than in the morning. The summation of the sine functions (Fig. 2A, dashed lines) results in a smooth yearly radiation distribution around the trunk varying over a 40-fold range (Fig. 2B) with a maximum to the southeast and minimum to the north. The effect of atmospheric light scattering on this pattern was not determined. However scattering only would raise the northern minimum and decrease the southeastern maximum but it would not change their azimuth positions.

**Atranorin and norstictic acid respond to light in opposite ways.**—Irradiance values calculated for each position around the trunk circumference are combined with the norstictic and atranorin concentrations observed in all 83 thalli (Fig. 3A). The lichen distribution around the trunk was fairly uniform, except for two narrow strips without thalli (areas without dots in Fig. 3A) exposed west and north-north-west. The most obvious feature is the increase of norstictic concentration with decreasing irradiance. Less obvious, due to scatter and a generally lower range of values, is the opposite behavior of atranorin. The scattering of the data can be reduced by removing from the analysis younger thalli weighing less than 4 mg (Fig. 3B). In the subset of more mature thalli the trends become clearer and are indicated by the curves through the data. Atranorin and norstictic values from B plotted as a function of yearly light intensity, independent of thallus position on the tree are presented (Fig. 3C, D respectively). The linear fits are meant only to show the opposite trends of atranorin and norstictic and do not imply necessarily a linear correlation. The large difference in norstictic acid content between mature thalli facing north (low light) vs. south (high light) can be observed directly on intact thalli by SEM (Fig. 3E). The north-south gradient in compound concentration also was found in the *Parmotrema* from another tree (data not shown).

**DISCUSSION**

**Depside/depidione amounts reflect primarily synthesis, not degradation or loss.**—Although microbial action in diseased (Lawrey et al. 1999) and old lichens, or extreme heat through volcanic activity (Culberson et al. 1977) can degrade lichen compounds, depsides and depsidones are chemically stable in healthy lichens under normal environmental conditions (Culberson et al. 1977). Decomposition products are rarely seen in fresh lichen extracts, in extracts stored for years on glass slides or in decades-old herbarium specimens. Decomposition requires harsh temperature and pH treatments in the laboratory. Compound stability is not only a function of chemistry but also of location; depsides and depidones accumulate extracellularly as water-insoluble accretions embedded in the polysaccharide matrix on the surface of fungal hyphae, not subjected to intracellular metabolic turnover and not easily removed by moisture or rain. In fact compound amounts as high as 5–10% of thallus dry weight are common (Huovinen and Ahti 1986a, b) and amounts higher than 25% have been reported (Ahmann and Mathey 1967). We therefore assume that the rates of degradation and loss, although not zero, are negligible in healthy lichens under normal conditions and that the amount present per unit weight in a thallus is primarily a function of the compound’s average biosynthetic rate over that thallus’ lifespan. This assumption finds in vivo support in studies showing that the amount of depsides and depidones tends to increase with thallus age (Stephenson and Rundel 1979, Asplund and Gauslaa 2007). This applies only to lichen depsides and depidones and not necessarily to other secondary metabolites.

**The exposed tree trunk: a natural setting with a yearly gradient of light distribution.**—Atranorin correlates directly, and norstictic inversely, with the calculated light intensities (Fig. 3). This suggests that on exposed vertical trunks absorbed light is the primary positional variable affecting the thalli colonizing them. We focused on yearly light exposure because the metabolic response to it is relatively slow because lichen compounds accumulate over months (Solhaug and Gauslaa 1996, Bjerke et al. 2002, McEvoy et al. 2006). The scatter in the data can be rationalized by viewing each thallus as a sensor that integrates light exposure effects over long periods and accumulates compounds as it grows (see preceding section); very young thalli initially would be “set” to produce low amounts of norstictic acid irrespective of exposure, and production rates would stabilize at exposure-appropriate levels as thalli grow older. Thalli of different ages are mixed at each exposure level, thus producing scatter (Fig. 3A). The scatter in fact decreases if age distribution is narrowed by removing younger thalli from the analysis (Fig. 3B). Other sources of scatter could be health differences among individual thalli, as well as small differences in lobe orientation relative to the sun and bark. The collective effect of these variables however is not sufficient to obscure the overall irradiance-dependent pattern described here. We will argue that directional radiation absorption regulates depside and depidione accumulation by selectively affecting temperature and water potential in each thallus.

**The case for temperature/moisture as effectors of depside**
Fig. 3. Secondary compound concentration and light exposure in a Parmotrema population. In panels A and B the left axes represent the light distribution (dashed line; scale as in Fig. 2B); the right axes represent compound concentration (open circles, norstictic acid; closed circles, atranorin). The 0–360° position on the x axis is shifted to the south (relative to that in Fig. 2) to better visualize the norstictic acid pattern; each symbol’s x coordinate represents the position of the corresponding thallus on the tree trunk. Panel A: light exposure and compound distribution in all 83 thalli. Panel B: light exposure and compound distribution in mature thalli weighing more than 4 mg; the curves fitted through the compound data are 6th order polynomials. Panels C and D: correlation in mature thalli between yearly light exposure and atranorin ($P_{\text{slope}} = 0.0120$) or norstictic acid ($P_{\text{slope}} = 0.0016$). The regression lines are meant to highlight the opposite light vs. compound correlations, not to suggest necessarily linearity. Panel E: scanning electron micrographs of cross sections through the medulla of thalli facing north (low light) and south (high light). Notice the richer norstictic accretions on the hyphal surfaces of the north-facing thallus. Bar = 10 μm.
and depsidone metabolism.—Light is important for fungi, where specific light receptors mediate its effects on growth, mating and pigment formation (Idnurm and Heitman 2005). Photosynthetic effect is essential to lichen fungi that depend on the carbon fixed by their photobionts (Palmqvist 2000, Palmqvist and Sundberg 2000). With regard to lichen secondary metabolism, a direct influence of light on anthraquinones such as parietin and melanins, common to many microorganisms in addition to lichens, is certainly compatible with the data (Solhaug and Gauslaa 1996, Nybakken et al 2004). However, direct light control of lichen-specific depsides, depsidones and dibenzofurans, for instance via receptors as hypothesized by Swanson and Fahselt (1997), remains experimentally inconclusive (see INTRODUCTION). On the other hand, thallus hydration mediates the effects of solar radiation, temperature and wind on lichen physiology, anatomy, morphology and ecology (Larson 1979; Kershaw 1985; Lange et al 1986; Kappen and Redon 1987; Pintado et al 1997, 2001; Gaio-Oliveira et al 2004). Thus it is plausible to assume that the response of depsides and depsidones to light also is regulated by the lichen’s water status. A posteriori support for this hypothesis can be derived from the following sources.

Under controlled conditions in the laboratory, decreases in water potential have been shown to favor the production of depsides, depsidones and usnic acid in cultured lichen mycobionts (Culberson and Armaleo 1992, Armaleo 1995, Hamada and Miyagawa 1995, Hamada 1996, Hamada et al 1996). In natural settings, a direct correlation between annual mean temperature and the concentration of the depsidone salazinic acid was reported for Ramalina siliquosa (Hamada 1981). In seminatural settings, a 3 C increase in temperature and a 16% decrease in humidity relative to ambient values resulted in 22-fold average increases for the depside gyrophoric acid in Peltigera didactila over 5 y (Bjerke et al 2003). These results parallel the increase of atranorin with increasing irradiance observed here.

However, the increase of norstictic with decreasing irradiance suggests that lower temperatures and higher water potential also should induce some compounds in cultured lichen fungi and lichens. Also this expectation has experimental support. In the cultured mycobiont of Ramalina siliquosa, production of the depside 4-O-demethylbarbatic acid increased with rising water potential in the medium (Hamada and Miyagawa 1995). In a landmark study with reconstituted Cladonia grown under controlled conditions Culberson et al (1983) showed that decreasing temperature up-regulated production of the depside barbatic acid and down-regulated that of the dibenzofuran didimic acid. Light intensities had no significant effect on either pathway at constant temperatures. In natural settings, Swanson et al (1996) reported a negative correlation between the amount of depsides in Umbilicaria americana and
monthly UV-B irradiance. The authors suggested that UV-B degraded the compounds. However, because UV-B irradiance increased in the warmer months, a downturn of compound biosynthesis with increasing temperature also seems possible.

Two different pathways respond differently to the same temperature-moisture cues.—The segregation of atranorin and norstictic in cortex and medulla, and their opposite responses to light (Fig. 4), suggest two distinct pathways activated differentially in the two tissues. Based on the characteristics of lichen polyketides (Fahselt 1994) and on the depside-depsidone biosynthetic connection proposed by Elix et al (1987) reasonable inferences can be made on these pathways (Fig. 5). Different regulatory components for the two sets of genes could mediate the divergent responses to the same internal temperature and water potential cues in each thallus. We cannot distinguish here between the individual contributions of thallus temperature vs. water potential to secondary metabolism. We use the temperature/water potential ratio $T/\Psi$ to express the inverse relationship generally linking these two variables under natural conditions, not as a strictly quantitative formulation (Fig. 5). In Parmotrema an increase in the overall $T/\Psi$ values at higher yearly irradiances stimulates the production of atranorin in the cortex. Conversely, a decrease in the overall $T/\Psi$ values at lower yearly irradiances stimulates norstictic production in the medulla (Figs. 3–5).

Wind, light and quantitative compound variability.—Air currents strongly affect evaporation and lichen water content (Larson 1979) and thus we predict that wind patterns will affect depside and depsidone
metabolism. In our area there is a yearly excess wind flow from the southwest (http://sti.srs.gov/fulltext/tr2002515/tr2002515.pdf). Yet this imbalance shows no obvious correlation to the directional changes in compound levels, while light does (Fig. 3A, B). This probably is due to the fact that around exposed trunks air turbulence erases any vectorial effects of winds, thus leaving light as the primary directional variable. The directional effects of light are discernible on exposed trunks due to their geometry but are superimposed on the evenly distributed effects of the moisture and temperature variation in the air surrounding the trunk (Fig. 5). In settings other than exposed trunks, directional effects of light would not necessarily be obvious and the relative weights of air moisture, wind and radiation on compound accumulation would depend on each microhabitat, as well as on thallus anatomy and morphology, that play a central role in water retention and evaporation (Larson 1979). The often noted thallus to thallus variability in compound concentration within a species therefore can be ascribed to each thallus’ differential exposure to light, wind and moisture (Fig. 5), functions of its placement on branches, rocks and soil.

On the adaptive roles of depsides and depsidones.—Allelopathic defense and light screening are the adaptive functions most commonly ascribed to lichen compounds (Fahselt 1994, Hueck 1999). In our view however, the divergent responses of cortical vs. medullar compounds to the lichen’s water status suggest distinct roles connected to the latter. In fact, the earliest proposed function for the water-repellent lichen compounds was to prevent excess water from flooding the medullar air spaces to maintain efficient metabolism. In our area there is a yearly excess wind flow from the southwest (http://sti.srs.gov/fulltext/tr2002515/tr2002515.pdf). Yet this imbalance shows no obvious correlation to the directional changes in compound levels, while light does (Fig. 3A, B). This probably is due to the fact that around exposed trunks air turbulence erases any vectorial effects of winds, thus leaving light as the primary directional variable. The directional effects of light are discernible on exposed trunks due to their geometry but are superimposed on the evenly distributed effects of the moisture and temperature variation in the air surrounding the trunk (Fig. 5). In settings other than exposed trunks, directional effects of light would not necessarily be obvious and the relative weights of air moisture, wind and radiation on compound accumulation would depend on each microhabitat, as well as on thallus anatomy and morphology, that play a central role in water retention and evaporation (Larson 1979). The often noted thallus to thallus variability in compound concentration within a species therefore can be ascribed to each thallus’ differential exposure to light, wind and moisture (Fig. 5), functions of its placement on branches, rocks and soil.

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**LITERATURE CITED**


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