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Light Exposure Affects Secondary Compound Diversity in Lichen Communities in Monteverde, Costa Rica

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ABSTRACT

Most lichen produce secondary compounds that have a variety of functions, including pathogen resistance, deterrence of herbivory, and protection from irradiance. In lichen, production of a given secondary compound is a species-specific trait. Thus, community composition may be strongly affected by ultraviolet light exposure, since certain species are able to produce UV-screening compounds while others cannot. To determine the effect of UV exposure on lichen communities, lichen morphospecies were sampled in pasture, forest edge, and forest interior environments and assayed for the presence of UV-absorbing secondary compounds. The Shannon-Weiner diversity index of UV-screening compounds was significantly higher in the pasture ($H' = 1.98$) than in the forest edge ($H' = 1.60$) ($t = 2.79$, $p < 0.05$) than in the forest interior ($H' = 1.60$) ($t = 3.66$, $p < 0.05$). However, the forest edge and interior communities did not differ significantly from one another with respect to diversity of UV-protective compounds ($t = 0.01$, $p > 0.05$). This is persuasive evidence that UV exposure is a significant factor in determining the species composition of lichen communities.

RESUMEN

La mayoría de los líquenes producen químicos secundarios que tienen una variedad de funciones, incluyendo resistencia a los microorganismos, resistencia a los herbívoros, y protección de los rayos del sol. Las especies de líquenes pueden ser identificados por los químicos que contienen, porque cada especie produce una sustancia o un grupo de sustancias específicos. Por consiguiente, es posible que la composición de las comunidades de líquenes sea afectado por la luz ultravioleta (UV), porque algunas especies pueden producir los químicos que absorben los rayos UV y otras no. Para determinar el efecto de los rayos UV en las comunidades de líquenes, las "morfoespecies" de líquenes fueron analizadas en los pastizales, en el borde del bosque, y en el interior del bosque para encontrar químicos que absorben UV. El Shannon-Weiner índice de diversidad de químicos que protegen contra los rayos UV fue más alta en los pastizales ($H' = 1.98$) que en el borde del bosque ($H' = 1.60$) ($t = 2.79$, $p < 0.05$) y también que en el interior del bosque ($H' = 1.60$) ($t = 3.66$, $p < 0.05$). Sin embargo, las comunidades en el borde y en el interior del bosque no se diferenciaron con respecto a la diversidad de los químicos que absorben UV ($t = 0.01$, $p > 0.05$). Eso es evidencia muy fuerte que la exposición a los rayos UV es importante para determinar la composición de especies en comunidades de líquenes.

INTRODUCTION

Lichen consists of a fungus, or mycobiont, in a symbiotic relationship with an algae or cyanobacteria, the photobiont. The mycobiont receives carbohydrate from its partner, while the photobiont, which is normally able to live only in aquatic or very moist habitats, can colonize harsher areas because it is protected by the fungus (Purvis 2000). Most lichen produces a wide array of energetically expensive secondary compounds. These chemicals have a variety of functions, including pathogen resistance, deterrence of herbivory, inhibition of seed or bryophyte spore germination, and regulation of the symbiotic association between the fungus and photobiont (Lawrey 1986). Another important role for lichen secondary compounds is protection from intense irradiance,

especially in the ultraviolet spectrum. Chemicals implicated in UV protection in lichen are depsides, depsidones, some β -orcinol dibenzyl esters, usnic acid, xanthenes, and pulvinic acid derivatives. For example, depsides and depsidones, secondary compounds unique to lichen, have very strong absorbance in the ultraviolet spectrum (Hale 1956). Lichen have been shown to increase their depside concentrations in response to increasing light exposure, suggesting that these compounds may have a photo-protective role (Culberson et al. 1983; Stepanenko et al. 2002). Aromatic lichen substances, such as the dibenzyl ester barbatolic acid, also absorb UV radiation (Mateos et al. 1991; Huneck 1999). Lichen production of usnic acid, yet another UV-absorbing compound, is correlated with level of irradiance (Bjerke et al. 2002). Pigments such as xanthenes and pulvinic acid derivatives also seem to serve as light screens (Brodo et al. 2001).

The composition of lichen communities is influenced by a variety of factors, including water availability, temperature, substrate, and light (Umaña and Sipman 2002). Presumably, a lichen's ability to protect itself from high levels of visible light and ultraviolet radiation will influence the habitats it is able to occupy. A study performed in West Greenland showed that lichen species with high concentrations of usnic acid inhabit more light-exposed areas than species with lower levels of this compound, since they are more protected from the harmful effects of intense irradiance (Bjerke and Dahl 2002). In a survey of lichen distribution in Thailand, lichen containing red and yellow pigments (associated with both UV screening and protection from xerophytic conditions) were found in deciduous dipterocarp forest and high-altitude montane oak forest, but not in seasonal evergreen forest or tropical mixed deciduous forest. The authors concluded that this pattern could be explained by the differing light intensities, moisture levels, and fire regimes among the four types of forest (Wolseley and Aguirre-Hudson, 1997).

In lichen, production of a given secondary compound is a species-specific trait; lichen can be identified to species with a combination of morphological descriptions and chemical spot tests that react with specific lichen substances (Brodo et al. 2001). Community composition may be strongly affected by ultraviolet light exposure, since certain species are able to produce UV-screening compounds while others cannot. However, the effect of ultraviolet light levels on lichen communities in the tropics has not been well researched. This study aimed to determine if UV light exposure is a significant factor in determining the makeup of lichen communities in forest ecosystems. The prevalence and types of UV-absorbing compounds were expected to differ among lichen communities in pasture, forest edge, and forest interior environments, since light exposure decreases in intensity from open areas to closed canopy forest.

MATERIALS AND METHODS

This study was performed on and around the property of the Estación Biológica in Monteverde, Costa Rica, an area located in a Lower Montane Wet Life Zone (*sensu* Holdridge 1947). Nine trees were selected in each of three habitats (communities): pasture, forest edge, and forest interior. The individuals chosen were of similar diameter (pasture: 85.1 ± 5.23 cm; edge: 83.9 ± 10.9 cm; interior: 92.6 ± 14.1 cm). To ensure that light intensities in the understory differed for trees in the forest edge and those in the forest interior, a canopy densitometer measured canopy cover as a proxy for light levels. Average canopy cover was $89.6 \pm 3.71\%$ at edge sites compared to $96.1 \pm 1.54\%$ at forest sites. All lichen from the ground to a height of two meters was identified to

morphospecies, and approximately one cm² of material was collected from every morphospecies on every tree.

Lichen samples were assayed using spot tests for the presence of secondary compounds. Three reagents were used: 10% potassium hydroxide, undiluted bleach, and 20% Lugol's solution in a pH 11 buffer; a fourth test required application of potassium hydroxide followed by addition of bleach. Using a capillary tube, small amounts of each reagent were applied to the outer surface, or cortex, of every lichen. Three-dimensional foliose (leafy) and fruticose (branching) lichen were sliced with a razor to expose the medulla, or inner layer of hyphae, so that it could be tested as well. A dissecting microscope aided in detection of color changes following the addition of reagents.

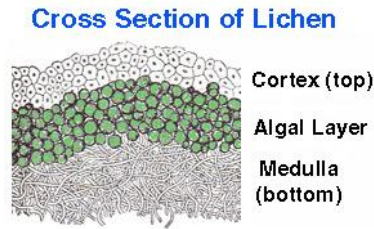


Figure 1. The layers of a lichen: the outer cortex, the algal layer (where the photobiont resides), and the medulla, composed of fungal hyphae (Fogel 2006). Foliose lichen may have a lower cortex as well.

The combination of color changes for each of the four tests indicated the specific secondary compound present in a given lichen sample; a key developed by Brodo et al. (2001) assisted in chemical identification. Because the color of the lichen cortex may obscure the results of the spot test, white tissue paper was used to blot the samples; the reacting secondary compounds bleed onto the paper to allow for easier identification (Susan Will-Wolf, personal communication).

To analyze diversity of UV-screening secondary compounds across habitats, the Margalef's richness index and the Shannon-Weiner diversity index were modified to fit the study. For the purposes of this analysis, S is the number of different UV-absorbing secondary compounds observed in a community, while N is the total number of photo-protective compounds observed in all the lichen samples in that habitat.

RESULTS

Two hundred and eight individuals representing 96 lichen morphospecies were sampled in pasture, 157 individuals of 88 morphospecies were collected in the forest edge, and 122 individuals of 72 morphospecies were found in the forest interior. In total, 189 morphospecies were identified. There was not a great deal of overlap in morphospecies composition among communities (Sorenson Index of Similarity, pasture and edge, $C_s = 0.228$; pasture and forest, $C_s = 0.262$; edge and forest, $C_s = 0.400$). Most morphospecies tested positive for UV-screening secondary compounds regardless of habitat: 88.5% in pasture, 88.6% in the forest edge, and 98.6% in the forest interior.

The richness of UV-protective compounds was higher in the pasture community than in either the forest edge or forest interior habitats (Table 1). Additionally, the Shannon-Weiner diversity of UV-screening compounds was significantly higher in the

pasture ($H' = 1.98$) than in the forest edge ($H' = 1.60$) ($t = 2.79$, $p < 0.05$) and then in the forest interior ($H' = 1.60$) ($t = 3.66$, $p < 0.05$). However, the forest edge and interior communities did not differ significantly from one another with respect to diversity of UV-protective compounds ($t = 0.01$, $p > 0.05$). In addition to determining abundance (N) of UV-screening compounds in the three communities, a modified N was calculated to describe the abundance of all chemical profiles in lichen morphospecies, including the presence of photo-protective compounds, the presence of compounds that did not absorb UV, and the absence of compounds. This was accomplished by multiplying the number of morphospecies by a correction factor to account for the individuals that proved to have more than one compound.

Table 1. Richness of UV-screening chemicals (S), UV-screening compound abundance (N), Margalef's richness index for UV-absorbing chemicals (S_{marg}), number of morphospecies (M), average number of compounds per morphospecies (C_{ave}), and modified chemical abundance (N_{mod}) in pasture, forest edge, and forest interior communities. Note that N_{mod} values were obtained by multiplying M and C_{ave} .

	Pasture	Edge	Interior
S	12	9	7
N	95	78	72
S_{marg}	2.42	1.84	1.40
M	96	88	72
C_{ave}	1.1	1.0	1.0
N_{mod}	106	89.0	73.0

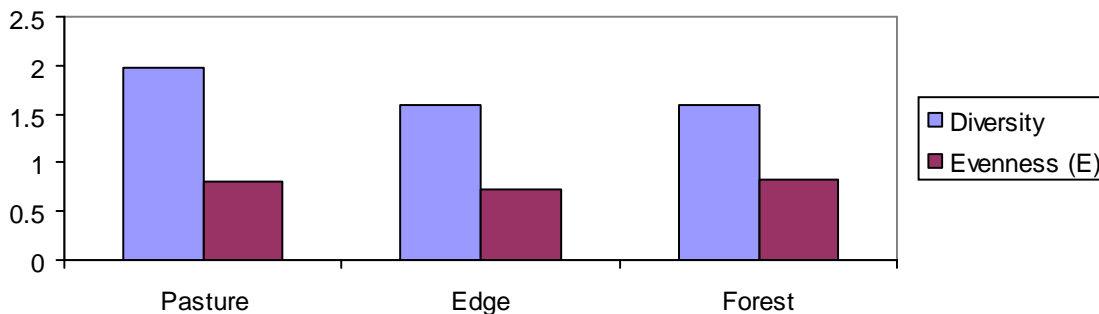


Figure 2. Shannon-Weiner diversity (H') and evenness (E) indices for UV-screening compounds in pasture, forest edge, and forest interior environments.

Variance about the mean chemical richness from tree to tree within pasture, edge, and forest environments was equivalent among habitats (Bartlett Test, $F = 0.789$, $p = 0.454$). Intrahabitat variation was also equivalent among pasture, edge, and interior with respect to the mean UV-protective compound diversity (Bartlett Test, $F = 1.01$, $p = 0.363$) and the mean evenness (Bartlett Test, $F = 1.21$, $p = 0.297$).

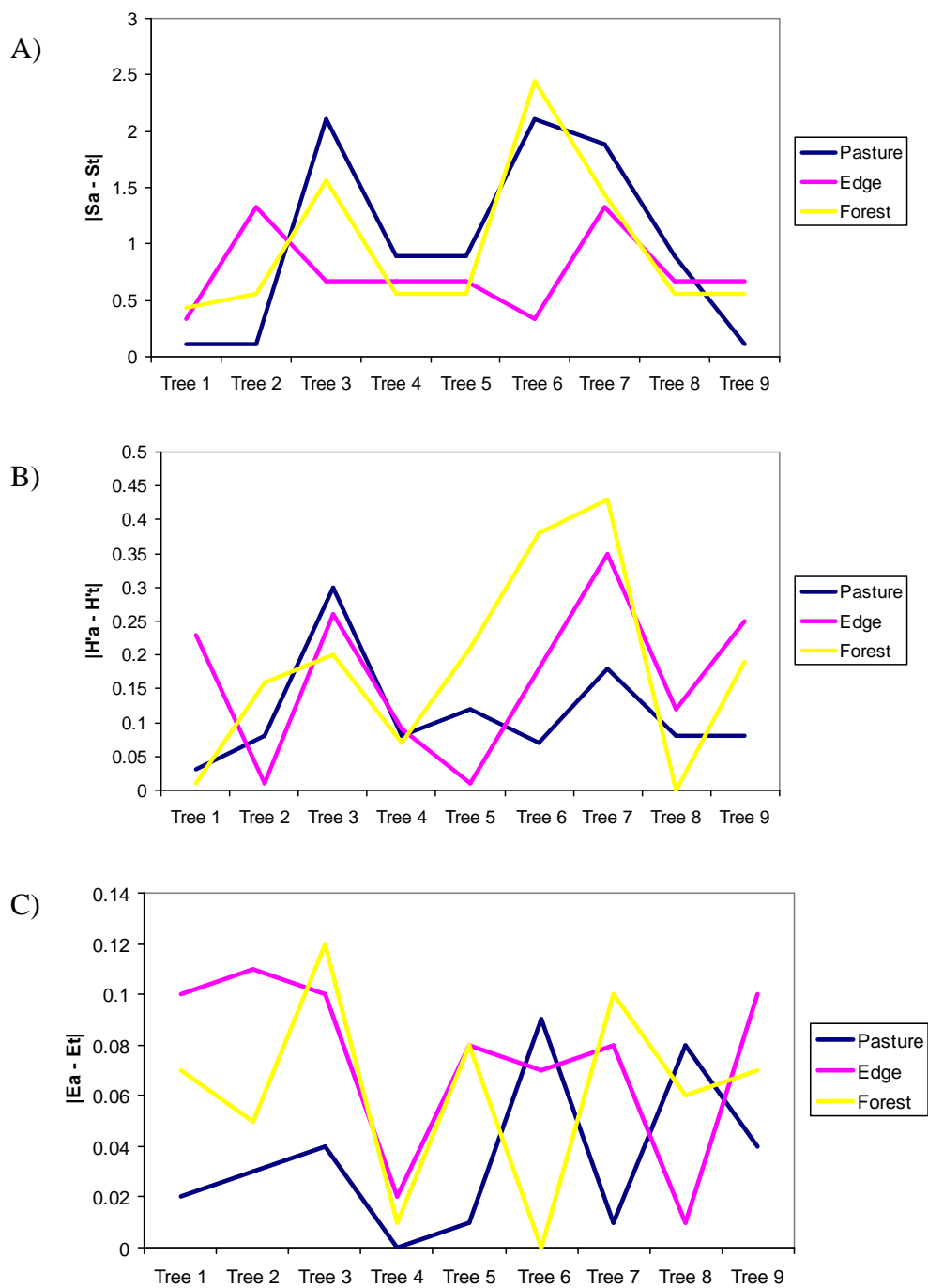


Figure 3. Distance from the habitat-specific mean of each of three diversity parameters for trees in pasture, forest edge, and forest interior. For S , H' , and E , variability of trees within a habitat was similar among all three communities. A) The difference between average compound richness (S_a) and compound richness for individual trees (S_t) in each habitat. B) The difference between average H' of compounds (H'_a) and compound H' for each tree (H'_t) in the three habitats studied. C) The difference between average E (E_a) and the tree-specific E (E_t) in pasture, edge, and interior.

DISCUSSION

In contrast to predictions, the percentage of UV-protected lichen morphospecies in a community did not decrease with decreasing light intensity. In fact, the forest interior community had the highest proportion of morphospecies containing light-screening chemicals, though all three habitats contained a very high percentage of species with ultraviolet-absorbing compounds. This may indicate that lichens require UV screens at all levels of light exposure. However, the richness and diversity of photo-protective secondary compounds were higher in the pasture than in the forest edge or interior; many of the UV-screening compounds found in pasture morphospecies were not observed in either forest environment. Since variance in diversity indices among trees within each community was similar in pasture, forest edge, and forest interior, the high compound diversity in pasture is not a statistical artifact of one or a few extremely compound-rich individuals, but a real trend. Furthermore, the average number of compounds per lichen was very low in all habitats. Thus chemical diversity in pasture can be attributed to a more chemo-diverse community structure rather than the presence of a few lichen morphospecies with multiple UV-screening compounds.

It is likely that different secondary compounds incur varying energetic costs to the lichen that produce them; the ability of chemicals to effectively absorb most wavelengths of UV may also differ. Perhaps the compounds observed in the pasture but not in the forest are more “expensive” yet also more efficient at blocking ultraviolet radiation. Since pasture trees are more directly exposed to sunlight, the extra resources devoted to the production of highly effective UV-absorbing compounds would be well worth the investment. Though light exposure (as measured with a canopy densitometer) did differ between forest edge and forest interior environments, compound diversity did not. Perhaps lichen communities must experience a threshold level of light intensity before it becomes adaptive to begin producing more expensive UV-protecting substances.

Light levels are not the only abiotic factors that change between forest and pasture environments. More shaded areas are moister and cooler; furthermore, humidity, temperature, and light levels are more stable in forest than exposed habitats. There are also differences in the biota of forest interiors, forest edges, and open areas. When examining such differences, it is important to realize that lichen secondary compounds may have multiple functions (Huneck 1999), which might partly explain the discrepancy in compound diversity between forest and pasture environments. While the pasture contained UV-screening chemicals found nowhere else, each of the compounds observed in forest interior lichen was also found in the pasture community. Furthermore, only one morphospecies in the forest edge contained a chemical not found in the pasture. Perhaps the compounds exclusive to the pasture not only protect from irradiance, but help the lichen there adapt to greater environmental variability of moisture and temperature. Though of course the lichen communities on forest edge and interior trees face their own specialized set of selective pressures, this was not evidenced by a set of compounds unique to the forest habitats. Perhaps the chemicals that are more useful in forest environments (e.g. herbivory deterrents) are not detectable with spot tests. This is the case for fatty acids, which do not react with potassium hydroxide, bleach, or Lugol's solution, and that are useful in repelling water to provide air spaces for gas exchange.

Such compounds would most certainly be more adaptive in the forest edge or interior than in the pasture.

Clearly, more research is required to determine the exact biological roles of lichen substances. Elucidating the specific functions of various lichen secondary compounds would help provide a better picture of community dynamics in response to light exposure. Clarifying the chemical pathways for UV-screening compounds would also assist in identifying those that may be more energetically expensive to produce.

Nevertheless, the fact remains that the diversity of compounds that are known to absorb UV was highest where irradiance is strongest. This is persuasive evidence in favor of the view that UV exposure impacts lichen community structure by providing a selective pressure that favors those species with the ability to produce UV-protective compounds. Therefore, altering light intensities within a forest (e.g. through fragmentation or selective deforestation) may alter lichen community composition if the change in the light regime is sufficiently dramatic.

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