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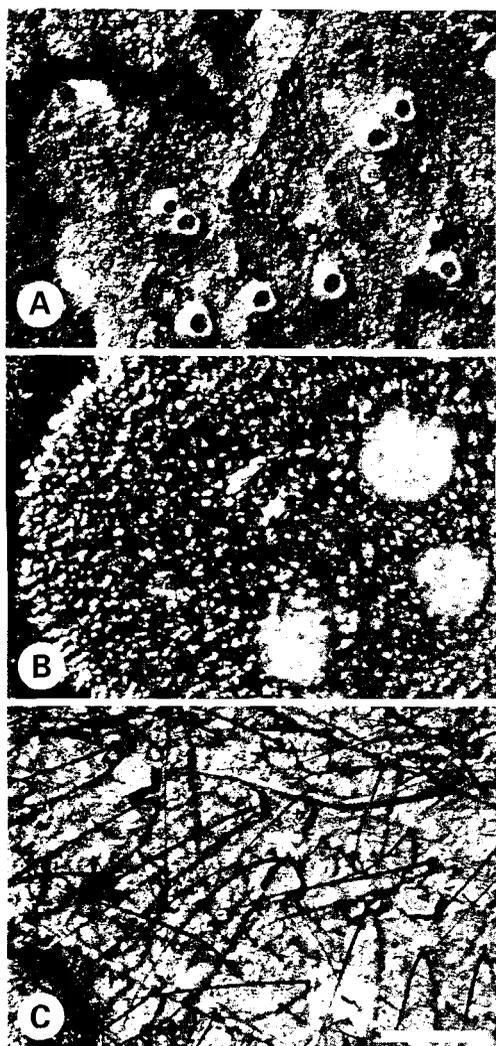


FIG. 1. Foliicolous lichen thalli showing the verrucose surface indicating deposition of calcium oxalate crystals. A, *Calenia triseptata*; B, *Echinoplaca strigulacea*; C, *Tricharia carneae*. Scale=1 mm.

Method

Standards of calcium oxalate, oxalic acid and magnesium hydroxide were obtained from Aldrich and used without further purification. Magnesium oxalate was prepared by the direct reaction between equimolar quantities of oxalic acid and magnesium hydroxide aqueous solutions; the white solid was filtered, washed several times with cold water and dried.

Good quality Raman spectra were obtained using a Bruker IFS 66 spectrometer with a FRA 106 Raman module attachment and Nd³⁺/YAG laser excitation at

1064 nm. This will be referred to as the macroscopic system. To prevent sample degradation, laser powers between 10 and 30 mW were used and up to 4000 spectral scans were accumulated with a 4 cm⁻¹ resolution with a laser footprint of about 100 µm at the sample. A series of spectra was examined to ensure that longer accumulation times did not result in spectral degradation. Raman spectra were also obtained using the dedicated micro-Raman system, comprising the Bruker IFS 66 system coupled with a Nikon microscope fitted with CCD camera and ×40 objective. This microscopic system results in a laser footprint of about 20 µm at the sample.

It was found that the use of near-infrared excitation was essential for the observation of the Raman spectra from the foliicolous lichens since visible excitation with radiation at 633 nm was subject to extensive fluorescence emission background which swamped the Raman signals. This is quite a common occurrence in the area of biological Raman spectroscopy.

Results and Discussion

Interpretation of Raman spectra

The macroscopic Raman spectra of the three lichen species investigated are shown in Fig. 2A. The best quality Raman spectrum was obtained from *Echinoplaca strigulacea*; therefore most of the discussion on vibrational assignments is based on a comparison of this spectrum with the standards of calcium oxalate and magnesium oxalate (Fig. 2B). The Raman band frequency values and tentative vibrational assignments are provided in Table 1.

The first and probably the most important question concerns the chemical composition of lichens on leaf substrata. In previous studies we have noted the presence of hydrated calcium oxalate in the encrustations of lichens on calcium-rich substrata (Edwards *et al.* 1991*b*, 1992, 1997*a*), and in other studies we found evidence for the production of calcium oxalate monohydrate in lichens growing on silicious substrata (Edwards *et al.* 1994, 1997*b*). In this latter case, it was concluded that the calcium was obtained from airborne material.

The spectrum of *Echinoplaca strigulacea* shows two bands, one of high intensity at 1463 cm⁻¹ and another of medium intensity at 1490 cm⁻¹, that are vibrational modes characteristic of calcium oxalate.

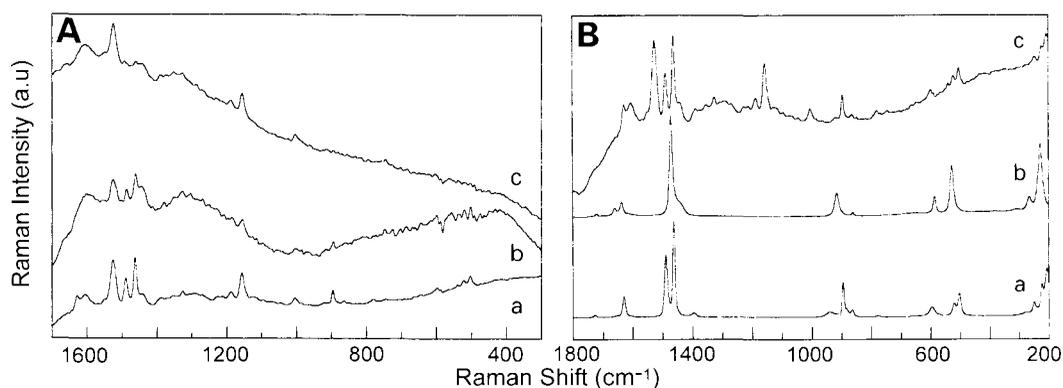


FIG. 2. Macroscopic Fourier transform Raman spectra. A, *Echinoplaca strigulacea* (a), *Calenia tripsetata* (b) and *Tricharia camea* (c) excited at 1064 nm; B, *Echinoplaca strigulacea* (c) compared with spectra of standards of calcium oxalate monohydrate (a) and magnesium oxalate dihydrate (b).

TABLE 1. Raman wavenumbers of hydrated calcium and magnesium oxalates and of three foliicolous lichens from Costa Rican tropical forests

Wavenumber (in cm^{-1})					Vibrational assignments
Calcium oxalate	Magnesium oxalate	<i>Echinoplaca</i>	<i>Calenia</i>	<i>Tricharia</i>	
1726	1721				$\nu(\text{C}=\text{O})$
	1660				$\nu(\text{C}=\text{O})$
1629	1637	1625			$\nu(\text{OCO})$
		1605	1602	1606	Chlorophyll
		1526	1521	1526	$\nu(\text{C}=\text{C})$ carotenoids
1490		1490	1488	1494	$\nu(\text{OCO})$
1463	1472	1463	1460	1461	$\nu(\text{OCO})$
		1442 sh	1444 sh	1445 sh	
1397				1353	
		1327	1328	1331	Chlorophyll
		1289			Chlorophyll
		1187		1189	
		1157	1156	1157	$\nu(\text{C}-\text{C})$ carotenoids
		1005	1003	1004	Aromatic $\nu(\text{C}-\text{C})$
942					
	917	910			
896		896	895		$\nu(\text{CC})$
865	863	867		843	$\nu(\text{CC})$
		744	747	746	Chlorophyll
597	587	598	596		$\rho(\text{OCO})$
522	528	521	517		$\delta(\text{OCO})$
504					$\delta(\text{OCO})$
250	266				
223	228				

Other bands at 896, 865, and 522 cm^{-1} of lower intensity are also due to several vibrational modes of calcium oxalate (Bickley *et al.* 1991; Edwards *et al.* 1991a; Edwards & Lewis 1994). Hydrated calcium oxalate can occur in two different forms, namely

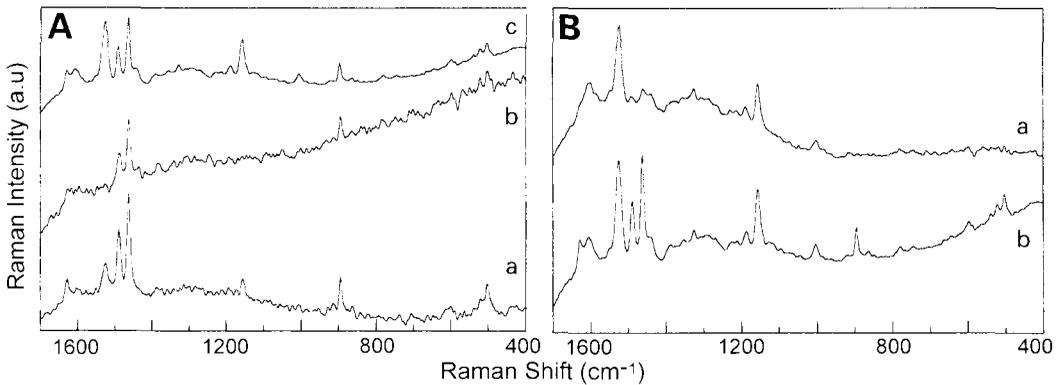


FIG. 3. Fourier transform Raman spectra. A, spectra obtained with the microscope ($\times 40$ lens) of *Echinoplaca strigulacea* (a) and *Calenia triseptata* (b) compared with the macroscopic spectrum of *Echinoplaca strigulacea* (c); B, macroscopic spectra of *Echinoplaca strigulacea* (b) and the substratum (leaf) on which it grows (a).

monohydrate (whewellite) and dihydrate (weddelite), which can be distinguished by observing the wavenumber of the carbon-oxygen stretching mode in the Raman spectra (Edwards & Lewis 1994). In the monohydrate form this vibrational band can be observed at 1493 cm^{-1} , whereas for dihydrate it can be expected at 1476 cm^{-1} . Analysis of the data obtained clearly shows that the calcium oxalate present in the investigated lichens is the monohydrate form.

In view of their unique substratum, the question arises as to the production of calcium oxalate by foliicolous lichens, when other elements derived atmospherically or known to be present in the substratum have the potential to produce oxalates. The Raman spectra of several metal oxalates have been recorded in our database and it is possible to discriminate between different metal counter-ions from their characteristic spectra; for example, the Raman spectrum of magnesium oxalate (shown in Fig. 2B), with a single band at 1472 cm^{-1} , was not present in any of the spectra obtained, but it was possible to attribute unequivocally several of the Raman bands to calcium oxalate in the spectra of *Echinoplaca strigulacea* and *Calenia triseptata*. On the other hand, the Raman spectrum of *Tricharia carnea* shows some bands with very low intensity in the region between $1450\text{--}1500\text{ cm}^{-1}$, and here the

assignment is not so clear; an explanation of this observation could be that the production of calcium oxalate is less significant for this system than for the other two species studied.

However, other vibrational bands present in the macroscopic Raman spectra of the lichens show the presence of further chemicals; for instance, the two intense bands at 1157 and 1526 cm^{-1} present in all three species investigated are characteristic of carotenes or carotenoids (de Oliveira *et al.* 1997). These bands were also observed by Schrader *et al.* (1999) in the FT-Raman analysis of plant leaves. The Raman bands at 747 , 1290 , 1330 and 1605 cm^{-1} can be assigned to chlorophyll (Schrader *et al.* 1999; Lutz & Mantele 1991). The appearance of these bands is probably due to a pre-resonance Raman effect of chlorophyll when excited at 1064 nm (Mattioli *et al.* 1990). The observation of these bands is explained by the presence of the photobiont of these lichens, in all cases a species of *Trebouxia* or a closely related genus, and the presence of chlorophyll and accessory pigments in the leaf substratum.

The spectra of *Echinoplaca strigulacea* and *Calenia triseptata* obtained with the Raman microscope can be seen in Fig. 3A; for comparative purposes, the macroscopic Raman spectrum of *E. strigulacea* is also depicted. Analysis of Fig. 3A clearly shows

that in the case of *C. triseptata*, the spectrum obtained with the microscope only has bands at 1488, 1460 and 895 cm^{-1} which are very characteristic of calcium oxalate. However, the Raman spectrum of *E. strigulacea* shows other Raman bands, at 1155 and 1526 cm^{-1} , characteristic of carotenoids; Fig. 3B shows the Raman spectrum of the leaf substratum of *E. strigulacea*, from which it is clear that the bands at 1155 and 1525 cm^{-1} characteristic of carotenoids are present in the leaf substratum.

Ecological context

In a recent review of lichen activity associated with rocks and neogenesis of minerals, Adamo & Violante (2000) state that the massive presence of calcium oxalate in most species is probably due to a need for the lichen to dispose of an excess of calcium. However, Wadsten & Moberg (1985) claim that calcium oxalate occurs in lichens colonizing substrata such as bricks, wood and bark, where the calcium ion is not available. In previous Raman spectroscopic studies in our laboratory we have examined the encrustations of *Dirina massiliensis* forma *sorediata* on a variety of stone substrata and have identified the presence of calcium oxalate even on non-calcareous substrata (Edwards *et al.* 1997).

In the foliicolous lichens under study, the presence of calcium oxalate can reasonably be attributed to a reaction between oxalic acid produced by the mycobiont of the lichen with calcium ions derived from airborne dust and dissolved in rain falling on the forest canopy (Jordan *et al.* 1980; Veneklaas 1990). Since it is unlikely that an excess of calcium is present under such conditions, an alternative assumption might be the excretion and immobilization of oxalic acid, a terminal metabolite in fungal and plant tissues. However, the apparent correlation between the presence of calcium oxalate clusters or layers in the lichen thallus and the degree of exposure to light (Lücking 1997, 1998, 1999) also suggests an eco-morphological function, such as protection against high light intensities and damage of the photobiont cells.

There is some evidence from the literature that the production of calcium oxalate responds to varying light intensities, since this rather simple system suggests a very effective feedback mechanism: as long as light is the limiting factor, higher light intensities induce higher metabolic rates, and greater quantities of oxalic acid would have to be immobilized by excretion. In turn, the accumulating calcium oxalate crystals would then reduce light intensities by partial reflection. How these crystals are morphologically organized in the thallus, their possible morpho-ecological function, and the role of light in their production have yet to be fully explored.

Conclusions

The Fourier transform Raman spectra, with characteristic vibrational Raman bands at 1490 and 1463 cm^{-1} , obtained from *Calemia triseptata*, *Echinoplaca strigulacea* and *Tricharia carneae*, foliicolous lichens growing on leaves in the Costa Rican rainforests, show the significant presence of calcium oxalate monohydrate. In spite of the lack of calcium ions available via the leaf itself, this result can be compared with those described by Wadsten & Moberg (1985) showing the same behaviour in other lichen species on different substrata lacking calcium ions. One reasonable explanation for the presence of calcium oxalate in such quantities could be as a result of the incorporation of calcium ions from airborne dust and/or dissolved in rain falling on the forest canopy.

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REFERENCES

- Adamo, P. & Violante, P. (2000) Weathering of rocks and neogenesis of minerals associated with lichen activity. *Applied Clay Science* **16**: 229–256.
- Aptroot, A. & Sipman, H. J. M. (1997) Diversity of lichenized fungi in the tropics. In *Biodiversity of Tropical Microfungi* (K. D. Hyde, ed.): 93–106. Hong Kong: University Press.
- Bickley, R. I., Edwards, H. G. M. & Rose, S. J. (1991) A Raman spectroscopic study of nickel (II)

- oxalate dihydrate, $\text{Ni}(\text{C}_2\text{O}_4) \cdot 2\text{H}_2\text{O}$, and of dipotassium bis-oxalate nickel (II) tetrahydrate, $\text{K}_2\text{Ni}(\text{C}_2\text{O}_4)_2 \cdot 4\text{H}_2\text{O}$. *Journal of Molecular Structure* **243**: 341–350.
- Coley, P. D. & Kursar, T. A. (1996) Causes and consequences of epiphyll colonization. In *Tropical Forest Plant Ecophysiology* (S. S. Mulkey, R. L. Chazdon & A. P. Smith, eds): 337–362. London: Chapman & Hall.
- de Oliveira, L. F. C., Dantas, S. O., Velozo, E. S., Santos, P. S. & Ribeiro, M. C. C. (1997) Resonance Raman investigation and semi-empirical calculations of the natural carotenoid bixin. *Journal of Molecular Structure* **435**: 101–107.
- Edwards, H. G. M., Farwell, D. W., Rose, S. J. & Smith, D. N. (1991a) Vibrational spectra of copper (II) oxalate dihydrate, $\text{Cu}(\text{C}_2\text{O}_4) \cdot 2\text{H}_2\text{O}$, and of dipotassium bis-oxalate copper (II) tetrahydrate, $\text{K}_2\text{Cu}(\text{C}_2\text{O}_4)_2 \cdot 4\text{H}_2\text{O}$. *Journal of Molecular Structure* **249**: 233–243.
- Edwards, H. G. M., Farwell, D. W. & Seaward, M. R. D. (1991b) Raman spectra of oxalates in lichen encrustations on Renaissance frescoes. *Spectrochimica Acta, Part A* **47**: 1531–1539.
- Edwards, H. G. M., Farwell, D. W., Jenkins, R. & Seaward, M. R. D. (1992) Vibrational Raman spectroscopic studies of calcium oxalate monohydrate and dihydrate in lichen encrustations on Renaissance frescoes. *Journal of Raman Spectroscopy* **23**: 185–189.
- Edwards, H. G. M., Edwards, K. A. E., Farwell, D. W., Lewis, I. R. & Seaward, M. R. D. (1994) An approach to stone and frescoe lichen biodeterioration through Fourier Transform Raman microscopic investigation of thallus-substratum encrustations. *Journal of Raman Spectroscopy* **25**: 99–103.
- Edwards, H. G. M., Farwell, D. W. & Seaward, M. R. D. (1997a) FT-Raman spectroscopy of *Dirina massiliensis* f. *sorediata* encrustations growing on diverse substrata. *Lichenologist* **29**: 83–90.
- Edwards, H. G. M. & Lewis, I. R. (1994) FT-Raman spectroscopic studies of metal oxalates and their mixtures. *Spectrochimica Acta* **50A**: 1891–1898.
- Edwards, H. G. M., Russell, N. C. & Wynn-Williams, D. D. (1997b) Fourier Transform Raman spectroscopic and scanning electron microscopic study of cryptoendolithic lichens from Antarctica. *Journal of Raman Spectroscopy* **28**: 685–690.
- Edwards, H. G. M., Holder, J. M. & Wynn-Williams, D. D. (1998) Comparative FT-Raman spectroscopy of *Xanthoria* lichen-substratum systems from Temperate and Antarctic habitats. *Soil Biology and Biochemistry* **30**: 1947–1953.
- Elix, J. A., Lumbsch, H. T. & Lücking, R. (1995) The chemistry of foliicolous lichens 2. Constituents of some *Byssoloma* and *Sporopodium* species. *Bibliotheca Lichenologica* **58**: 82–96.
- Holder, J. M., Wynn-Williams, D. D., Rull Perez, F. & Edwards, H. G. M. (2000) Raman spectroscopy of pigments and oxalates *in situ* within epilithic lichens: *Acarospora* from the Antarctic and Mediterranean. *New Phytologist* **145**: 271–280.
- Jordan, C., Golley, G., Hallm, J. D. & Hall, J. (1980) Nutrient scavenging of rainfall by the canopy of an Amazonian rain forest. *Biotropica* **12**: 61–66.
- Komposch, H. & Hafellner, J. (2000) Diversity and vertical distribution of lichens in a Venezuelan tropical lowland rain forest. *Selbyana* **21**: 11–24.
- Lücking, R. (1997) Additions and corrections to the knowledge of the foliicolous lichen flora of Costa Rica. The family Gomphillaceae. *Bibliotheca Lichenologica* **65**: 1–109.
- Lücking, R. (1998) Ecology of foliicolous lichens at the Botarrama trail (Costa Rica), a neotropical rain forest site. II. Patterns of diversity and area cover and their dependence on microclimate and phorophyte species. *Ecotropica* **4**: 1–24.
- Lücking, R. (1999) Ecology of foliicolous lichens at the Botarrama trail (Costa Rica), a neotropical rain forest. IV. Species associations, their salient features, and their dependence on environmental variables. *Lichenologist* **31**: 269–289.
- Lücking, R. (2001) Lichens on leaves in tropical rain forests: life in a permanently ephemeral environment. *Dissertationes Botanicae* **346**: 41–77.
- Lücking, R. & Bernecker-Lücking, A. (2000) Lichen feeders and lichenicolous fungi: do they affect dispersal and diversity in tropical foliicolous lichen communities? *Ecotropica* **6**: 23–41.
- Lücking, R. & Lumbsch, H. T. (2001) New species or interesting records of foliicolous lichens, VI. *Sporopodium aeruginascens* (Lecanorales), with notes on the chemistry of *Sporopodium*. *Mycotaxon* **78**: 23–27.
- Lücking, R., Lumbsch, T. H. & Elix, J. A. (1994) Chemistry, anatomy and morphology of foliicolous species of the genera *Fellhanera* and *Badimia* (Lichenized Ascomycotina: Lecanorales). *Botanica Acta* **107**: 393–401.
- Lutz, M. & Mantele, W. (1991) Vibrational Spectroscopy of Chlorophylls. In *Chlorophylls* (H. Scheer, ed.). Boca Raton: CRC Press.
- Mattioli, T. A., Hoffmann, A., Lutz, M. & Schrader, B. (1990) Fourier-transform QY pre-resonance Raman spectroscopy of Bacteriochlorophyll-A. *Compte Rendu l'Académie des Sciences (Paris), ser. III* **310**: 441–446.
- Sanders, W. B. (2001) Preliminary light microscope observations of fungal and algal colonization and lichen thallus initiation on glass slides placed near foliicolous lichen communities within a lowland tropical forest. *Symbiosis* **31**: 85–94.
- Sanders, W. B. & Lücking, R. (2001) Reproductive strategies and relichenization observed *in situ* in leaf dwelling lichen communities. *Nature* (submitted).
- Santesson, J. (1970) Chemical studies on lichens. 28. The pigments of some foliicolous lichens. *Acta Chemica Scandinavica* **24**: 371–373.
- Santesson, R. (1952) Folliicolous lichens I. A revision of the taxonomy of the obligately folliicolous,

- lichenized fungi. *Symbolae Botanicae Upsaliensis* **12**: 1–590.
- Schrader, B., Klump, H. H., Schenzel, K. & Schulz, H. (1999) Non-destructive NIR FT Raman analysis of plants. *Journal of Molecular Structure* **509**: 201–212.
- Seaward, M. R. D. (1988) Contribution of lichens to ecosystems. In *Handbook of Lichenology*, Volume II (M. Galun, ed.): 107–129. Boca Raton: CRC Press.
- Seaward, M. R. D., Edwards, H. G. M. & Farwell, D. W. (1998) Fourier-transform Raman microscopy of the apothecia of *Chroodiscus megalophthalmus* (Müll. Arg.) Vězda & Kantvilas. *Nova Hedwigia* **66**: 463–472.
- Sérusiaux, E. (1989) Follicolous lichens: ecological and chorological data. *Botanical Journal of the Linnean Society* **100**: 87–96.
- Veneklaas, E. J. (1990) Nutrient fluxes in bulk precipitation and throughfall in two montane tropical rain forests, Colombia. *Journal of Ecology* **78**: 974–992.
- Wadsten, T. & Moberg, R. (1985) Calcium oxalate hydrates on the surface of lichens. *Lichenologist* **17**: 239–245.
- Wolf, J. H. D. (1995) Non-vascular epiphyte diversity patterns in the canopy of an upper montane rain forest (2550–3670 m), Central Cordillera, Colombia. *Selbyana* **16**: 185–195.

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