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Secretory organs: Implications for lipoid taxonomy and kerogen formation (seed ferns, Pennsylvanian, Canada)

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ABSTRACT

Secretory organs likely evolved with land plants in Silurian-Devonian time, but it is questionable if they were passed on to living cycadaleans upon the extinction of seed ferns (Triassic-Jurassic?). They are defined as ducts of schizo-, lysig- or rhexigenous origin that exuded a heterogeneous lipoid mixture that fossilized as secretory products (droplets). In this study we detailed (1) the physical properties and distributions based on the compression-preserved *Neuropteris ovata* var. *simonii* and *Laveineopteris rarinervis* frond sections (Late Pennsylvanian Sydney Coalfield, Canada). Examined were also 1300 cuticular slides representing a number of plant groups, complemented by published data to infer biomass accumulation as potential kerogen in the geological column. In addition, (2) from select pinnules of the two species mentioned, nine secretory products and four surrounding cuticles were analyzed by micro-FTIR to evaluate statistically (PCA) the chemotaxonomic potential, and the kerogen chemistry. Further studies in support of (1) or (2) included methods of NICI, SEM, TEM and AFM, and by EDS.

Results indicate that the secretory organs occurred in the entire *N. ovata* frond, its associate petiole and trunk, and in great abundance on the pinnules of both species in a random fashion. Secretory products show layering effects, surfaces can be intact, convoluted, folded, or damaged. At 100,000 magnification, microstructures are not observable. The exact chemical composition is unknown because of insolubility. IR spectra show peaks of functional groups frequently found in isocyanates, disubstituted alkynes, nitriles, polyynes, thiocyanates, and allenes, in part underpinned by EDS results. These compounds were presumably derived from the diagenetic alteration of resin-like terpenoid- or phenolic-related structures. That is particularly the case for the polyynes (i.e. 'polyacetylenes'), synthesized by living plants with a variety of biological functions, including pigments and flavorings, toxins, and chemical repellents.

The chemical information of the lipoid exudate, together with their morphological information and distribution will contribute to (i) the progress of chemotaxonomy and systematics of plant fossils, and (ii) a better understanding of the genesis of kerogens from plant-derived materials, particularly resinous remains.

1. Introduction

The study history of the black 'dots', 'punctuations' or 'Punktierung' on cuticles originated with certain Carboniferous seed ferns. Its history can be divided into two periods: 1867–1928 and 1929–2015. These coincide with progressive understanding of their nature due to advancing technical inventions, particularly in compound-light, scanning and transmission electron microscopies, Nomarski interference-contrast illumination (since 1955), and in infrared spectrometry.

For the period up to 1928, Bode (1929) summarized the interpretations of the black 'dots' as being, for example, sporangial seats, sori, hairy bases, *Spirorbis* sp., 'Eisenoxyde' (iron oxide), or fungal remains (Wang, 1997: fungal remains of Pl. 5, 2, 1997, and Kögel-Knabner et al., 1994, Fig. 2(d) are suspect of being secretory organs). In that seminal paper, Bode claimed that 'Drüsen' are not sporadic in [sic extant] plants '…

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sondern für ganz bestimmte Pflanzenarten charakteristisch ist.' He also established basic parameters upon which the present paper is built. These include their organic (chemical) nature by observing that after applying Schulze's process to coalified (compression) specimens of the seed fern *Mariopteris latifolia* Brongniart, the black 'dots' [and the cuticle] had survived this brutal oxidative treatment. From the experiments he hypothesized that (1) a fundamental chemical difference existed between the coal-compression matter and the 'dots', (2) 'dots' compare with 'Drüsen' (secretory organs) of the angiospermous species *Hypericum perforatum* or St. John's Wort (Bode, 1929, Fig. 1), and that (3) their chemical makeup comprises bitumen, oils, resins, or waxes; for short ethereal oils. In hindsight, he had described a secondary metabolite in fossilized form for the first-time.

For the period 1929–2015, 'dots' were of little interest to palaeobotanists, except observations by Leisman (1960) regarding the cells of the upper epidermis of *Callipteridium sullivanti*, that were usually filled with a dark substance similar to that found in the secretory canals, hence probably representing a 'gummy or resinous deposit', and by Barthel (1961, 1962). Litke (1966, p. 352, Pl. XIV, 1; XV) recognized the occurrence of 'extranuptialen Nektarien' in liana-type fossils in brown coal of Miocene age (20–5 Ma). Šimůnek (1996, Plate 1, Fig. 7) correctly identified 'exit pores', and Pryor (1990, Fig. 13) documented 'dot' preservation of *Neuropteris* Brongniart in coal balls. Krings (2000), on the other hand, discussed secretory cavities, accompanied by light photography, from the perspective of secondary metabolites, analogous with functions in extant flora. He argued against [palaeo] taxonomic utility, in the absence of analytical data from fossil flora.

This paper initiates a new period in the study of 'dots' for which we use the name secretory organs. In particular, those from the foliage of the two larger frond segments of the seed-ferns *Neuropteris ovata* (Hoffmann, 1827) var. *simonii* (Bertrand, 1930) Zodrow *et* Cleal 1998, and *Laveineopteris rarinervis* (Bunbury, 1847) Cleal et al., 1990, hereafter referred to as *N. ovata* and *L. rarinervis*, respectively, were investigated in detail. The analytical techniques include Nomarski interference-contrast illumination (NICI), scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), X-ray energy dispersive spectroscopy (EDS), and Fourier transform infrared spectroscopy (micro-FTIR).

Our contributions consist of (i) describing the physical nature and distribution of secretory organs in the two study species, assessing Carboniferous-Triassic occurrence/distribution based on cuticular analysis of 1300 thin sections housed at Cape Breton University, (ii) hypothesizing secretory organs as kerogen contributor to oil generation in sedimentary rocks (see D'Angelo et al., 2010), and (iii) showing their potential for lipoid chemotaxonomy based on chemometric arguments. We caution, however, that "… a relationship between source rock and rock kerogen cannot be directly established in most cases." (*in litt*., Dr. C. Eble, 2016).

At the same time, this study highlighted challenges of working with heretofore undetermined and poorly-documented fossilized organic material, although we considered models which included resins and essential oils (e.g., Lyons et al., 1982; Chadwick and Whelan, 1992; van Bergen et al., 1995; Tsubaki and Azuma, 2013; Adinew, 2014).

2. The data base, and sample preparation

2.1. The extensive data base for secretory organs

The senior author collected large numbers of Carboniferous compression-plant fossils from the Sydney Sub-Basin of the Maritime Carboniferous Basin (Figs. 1 and 2). This includes the largest known *N. ovata* frond segment of 65 cm, with cyclopteroid pinnules organically attached to an associate petiole and trunk from the Point Aconi Seam (Zodrow and Cleal, 1988, Pls. 2–4), and the 45-cm long bipinnate *L. rarinervis* specimen from the Lloyd Cove Seam (Fig. 3A and B, respectively). Included in the collection are compression specimens from the Mabou Sub-Basin (Zodrow and Vasey, 1986), the Stellarton Sub-Basin (Lyons et al., 1997; Zodrow et al., 2000), and the Bay St. George Sub-Basin (Zodrow et al., 2000; Bashforth, 2005), see Fig. 1. Specimens of Indiana 'paper' coal (DiMichele et al., 1984), *Dicroidium* flora of Argentina (D'Angelo et al., 2011), and extant foliar and ovular specimens of *Cycas rumphii* Miquel, and ovular specimens of cycadealean *Zamia furfuracea* L. filius, and *Encephalartos ferox* (Bertol) are also part of the collection.

From these specimens, the senior author had prepared ca. 1260 representative cuticular glass-mounted slides. With the addition of 40 cordaitean slides (Šimůnek, 1996), the data base comprised 1300 slides, curated at Cape Breton University. By far the largest number of slides contain cuticles from pinnules and other organs of seed ferns, followed by cordaiteans, and distantly by ferns and lycopsid. Other organs (160 slides) refer to the constituents of macerated trigonocarpalean ovules and cuticles associated with, but not attached to medullosalean fronds (Cleal et al., 2010; Zodrow et al., 2013, 2014) that were used to further test the distribution of secretory organs in the [female] reproductive organ of seed ferns. From living cycadealean foliar and ovular materials (80 slides) were also included to test for the presence of secretory organs in the presumptive closest living relative to the extinct medullosaleans (cf. Zodrow and D'Angelo, 2014, p. 839). Routinely examined for secretory organs were ovules of *Zamia furfuracea* L. filius and *Encephalartos ferox* (Bertol). Results are tabulated in Supplementary material, Table 1.

2.2. Preservation and sample preparation

The *N. ovata* and *L. rarinervis* specimens were deposited in a fine-grained sandstone of basal Cantabrian age in a slowly subsiding coastal plain (Forgeron et al., 1986), forming the non-marine Sydney Sub-Basin (see Calder, 1998). A vitrinite-reflectance value of Ro% 0.75 points to conditions for favorable compression/coalification preservation for the secretory organs (cf. Hacquebard, 1984, Fig. 3).

Secretory organs specifically for the analytical experiments were obtained from macerating select *N. ovata* pinnules for 25–45 min, and *L. rarinervis* pinnules for seven hours. Sample locations are marked in Fig.3A and B, respectively. Since the secretory organs occurred internally (e.g., Fig. 4), separating cuticles into lower and upper surfaces was necessary to expose them for analyses by micro-FTIR, EDS, SEM, and AFM. For TEM, however, cuticles remained intact to be able to ascertain upper and lower cuticle/secretory organ boundaries in situ.

3. Analytical methods

Imaging and measurements of secretory organs were carried out at 250 or 500 magnification using a biological microscope equipped with a digitizing camera and NICI.

Micro-FTIR is a tool especially designed for obtaining chemical information from small objects, and the 10–117 μm diameter secretory organs fit well micro-FTIR capabilities (cf. Mastalerz and Bustin, 1993a; Smith, 1996, p. 157; Heredia-Guerrero et al., 2014; Chen et al., 2015). A Nicolet 6700 spectrometer connected to a Nicolet Continuum microscope operated in reflectance mode was used to generate the data. The microscope was connected to a liquid, nitrogen-cooled mercury-cadmium-telluride (MCT) detector. Micro-FTIR spectra were obtained at a resolution of 4 cm^{-1} over the range 4000 cm^{-1} to 600 cm^{-1} wavenumber, using a gold plate as background. An OMNIC program was used for spectral processing.

The interpretive aspect of the IR spectra entailed two complementary, commonly used approaches in our FTIR work: (1) qualitative and (2) quantitative. (1) Involved routine IR-peak assignments of functional groups (structural parts of molecules or moieties), as well as groups and classes of compounds (Table 1), following published sources, for example Wang and Griffiths (1985); Colthup et al. (1990); and Rochdi and Landais (1991). (2) Involved mathematical treatment applied to the digitized spectra to calculate 11 semi-quantitative IR ratios. The