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SHORT COMMUNICATION

# Plant secondary compounds in the canopy and understorey of a tropical rain forest in Gabon

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Given their difficulty of access, the canopies of tropical rain forests are considered a last frontier of biological/ecological research (Lowman & Nadkarni 1995). Climbing techniques are arduous and do not reach the tips of branches; towers, cranes and walkways limit the spatial exploration of the forest.

Our understanding of plant–herbivore interactions in the canopy, and our knowledge of the plant secondary compounds that mediate many of those interactions (and may have novel biological activities), have lagged behind studies in the understorey. Insect biomass and diversity are greater in the canopy (Stork 1988), yet foliar damage by herbivores is relatively low (Coley & Barone 1996). Hallé (1998) has argued that the exposure of canopies should result in high concentrations and diversities of compounds, either developmentally controlled or induced by light, wind, desiccation (Timmerman *et al.* 1984), and/or exposure to herbivores and pathogens (Tollrain & Harvell 1999). Yet, the evidence for increases in compounds relative to the understorey is meagre, primarily from colorimetric assays for tannins and total phenols (Coley & Barone 1996, Lowman & Box 1983). Here we show large increased concentrations and diversities of plant secondary compounds in four tree species from a rain forest in Gabon.

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We participated in the rain forest canopy expedition at La Makande Station, 0°20′S and 11°45′E in La Forêt des Abeilles in Central Gabon in February–March 1999, organized by Pro-Natura and Operation Canopée. Mature undamaged foliage without epiphylls was sampled from 10 trees each of four common species, both in the canopy and understorey: *Aucoumea klaineana* Pierre (Burseraceae), *Marquesia excelsa* (Pierre) Fries (Dipterocarpaceae), *Paraberlinia bifoliolata* Pellegr. (Fabaceae), and *Xylopia hypolampra* Mildbr. (Annonaceae). Vouchers were deposited at MPU. We sampled canopy foliage at a radius of 1 km from the camp, by a sled ('luge') suspended from the dirigible, and 1–3 m saplings of the same taxa in the understorey in the same area. Similar amounts of fresh leaf tissue were extracted with 100% ethanol for 48 h at 23 °C in the dark, and both transported to the United States for analysis.

We used high pressure liquid chromatography (HPLC) to separate leaf compounds and determine peak areas of the separated compounds in the extracts. A Hewlett-Packard (HP) Model 1090M HPLC equipped with a diode array detector, Hypersil microbore column (HP ODS, 5 μm, 100 × 2.1 mm) and guard column (HP ODS) was used for all analyses. We used a column temperature of 40 °C and a flow rate of 0.5 ml $min^{-1}$. Extracts were passed through a 0.2-micron filter before 10 μl of each sample was injected. Compounds were eluted with an acetonitrile (MeCN) : water solvent system, using a linear gradient program from 0 to 100% MeCN over 10 min, and remaining at 100% for 10 min. We monitored absorbance at 280 nm, suitable for most aromatic compounds.

We further analysed the extracts for nitrogenous compounds by atmospheric pressure chemical ionization mass spectrometry (Ikan 1991). Extracts were poured into brine twice and extracted with chloroform. The green organic extract was extracted with $H_2SO_4$ (2M; 2 × 20 ml) and the alkaloid-containing aqueous phase was made basic by adding NaOH (3M), then back-extracted with chloroform (4 × 10 ml). The organic extracts were directly infused into a Finnigan Navigator LC-PDA-MS.

Antimicrobial activities (UV-A induced and non-phototoxic) against *Staphylococcus aureus* (methicillin sensitive and resistant), *Bacillus subtilis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Mycobacterium phlei* were assayed with the same canopy extracts (Downum *et al.* 1991).

These four taxa represent families known for producing different classes of secondary compounds. Both the Burseraceae and Dipterocarpaceae are known for secondary products of the terpenoid pathway, the Fabaceae for alkaloids and non-protein amino acids, and the Annonaceae for acetogenins. The canopy samples from each species were dramatically increased (more than 4.4 times for numbers of compounds and their relative concentrations, Table 1). All of the detected compounds had aromatic moieties (based on the wavelength used for detection), and many were likely to be phenolic compounds. None of these

Table 1. Relative amounts and diversities of compounds detected by HPLC. Relative amounts of aromatic compounds based on the total integrated peak areas of their absorbances at 280 nm, and an optical density threshold of 0.3, additions of all detectable peaks, per unit mass (1 g dry). Estimates on a per area basis were very similar. Values are means ± S.E.s, and were compared by t-tests, with significance of *, $P < 0.05$; **, $P < 0.005$; and ***, $P < 0.0005$.

| Species | Canopy | Understorey | P |
|---|---|---|---|
| *Aucoumea klaineana* | | | |
| Number of compounds | 6.5 ± 0.3 | 1.4 ± 0.3 | *** |
| Concentration | 3378 ± 375 | 1220 ± 427 | * |
| *Marquesia excelsa* | | | |
| Number of compounds | 6.6 ± 0.3 | 1.6 ± 0.3 | *** |
| Concentration | 7116 ± 645 | 706 ± 153 | *** |
| *Paraberlinia bifoliolata* | | | |
| Number of compounds | 8.7 ± 0.9 | 1.8 ± 0.3 | *** |
| Concentration | 5616 ± 1256 | 1432 ± 442 | * |
| *Xylopia hypolampra* | | | |
| Number of compounds | 11.2 ± 0.6 | 2.9 ± 0.6 | ** |
| Concentration | 6777 ± 647 | 2928 ± 439 | ** |

compounds were nitrogenous, based on negative results from the electro-spray mass spectrometry. The greatest number of compounds (from the summary of each species sample) was produced from tree crowns; those exclusively from the crowns were half or more of the total number of compounds detected (Table 2). A few compounds were produced in the understorey only.

Extracts of canopy foliage of all taxa were antimicrobial (but not UV-A activated), especially *A. klaineana* and *P. bifoliolata* (Table 3). *Marquesia excelsa* extracts were less strongly antimicrobial, and those of *X. hypolampra* were only active for *Mycobacterium phlei*. The qualitative nature of these tests made it

Table 2. Total numbers of compounds detected by HPLC in understorey and canopy samples of the four rainforest trees.

| Species | Numbers of compounds produced | | | | |
|---|---|---|---|---|---|
| | Total | Crown | Crown only | Understorey | Understorey only |
| *Aucoumea klaineana* | 16 | 12 | 7 | 8 | 2 |
| *Marquesia excelsa* | 17 | 13 | 9 | 8 | 4 |
| *Paraberlinia bifoliolata* | 22 | 19 | 14 | 6 | 2 |
| *Xylopia hypolampra* | 34 | 27 | 22 | 12 | 7 |

Table 3. Results of bioassays against bacteria, without light or with exposure to UV radiation.

| Species | n | SamS | | SamR | | B.s. | | E.F. | | M.p. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | UV | dark | UV | dark | UV | dark | UV | dark | UV | dark |
| *Aucoumea klaineana* | 3 | +++ | +++ | +++ | +++ | +++ | +++ | ++ | ++ | +++ | + |
| *Marquesia excelsa* | 2 | ++ | ++ | ++ | ++ | – | – | – | – | – | – |
| *Paraberlinia bifoliolata* | 2 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| *Xylopia hypolampra* | 6 | – | – | – | – | – | – | – | – | ++ | ++ |

–, no inhibition zone; +, inhibition zone < 6 mm in at least half of samples; ++, inhibition zone 6–10 mm in all samples; +++, inhibition zone of more than 10 mm in all samples. Bacterial abbreviations are: SamS, *Staphylococcus aureus* methicillin sensitive; SamR, *Staphylococcus aureus* methicillin insensitive; B.s., *Bacillus subtilis*; E.f., *Enterococcus faecalis*; M.p., *Mycobacterium phlei*.

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unreasonable to compare the activities of extracts from the crowns with those from the understorey. However, we are presently working to determine the structures of the active components. Then we could determine the degree of antimicrobial activity in each foliage sample by the relative amount of that compound from HPLC.

The crowns of these rain forest trees produce significantly more secondary compounds at higher concentrations than understorey saplings. Some of the compounds are biologically active, and could help reduce damage from herbivory and disease. A variety of physical and biological factors may enhance their production in the canopy, although light may be the most important, particularly for products of the shikimate pathway. Frankel & Berenbaum (1999) showed that light increased the production of antioxidants in tropical rain forest plants, and Langenheim *et al.* (1991) showed a similar effect for terpenoid compounds in *Hymenaea* and *Copaifera*. High concentrations and diversities of these secondary compounds may help explain the relatively low levels of leaf damage from herbivory we observed in the canopy of the forest at La Makande (Downum, Hallé and Lee, unpubl. data) as well as other tropical forests (Coley & Barone, 1996, McKey *et al.* 1978).

The dramatic differences in structural diversity and levels of compounds suggest that surveys for biologically active molecules (i.e. new drugs or pesticides) should emphasize the canopy, and that estimates of the number and value of new plant-derived drugs could be increased (Balick *et al.* 1996). We collected the canopy samples in 4 h of flying time with the dirigible and sled. They are clearly the most effective means of access for such rapid surveys.

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