

# The Ecosystem of Microorganisms, Bees, and *Clusia* Floral Resin and Oils, from the Chemistry Point of View

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## INTRODUCTION: BOTANICAL ASPECTS

The neotropical genus *Clusia* L. (family Clusiaceae or Guttiferae) comprises about 250 species distributed from southern Florida to southern Brazil. The plants are woody and evergreen with often rather coriaceous and more or less carnosose leaves. Various life-forms occur in the genus: shrubs, small to medium-sized trees, hemi-epiphytes, and rarely lianas. In several species more than one life-form can be found, e.g., species which occur as hemi-epiphytes in dense forests may be found as shrubs or small trees in open vegetation forms. In some hemiepiphytic species additionally a strangling habit was observed, like in species of *Ficus* (Moraceae), where the aerial roots of an hemi-epiphyte enlarge around the host tree and eventually cause its death. The plants of many *Clusia* species occur under rather dry conditions, which might be responsible for the typical leaf texture. Eco-physiological investigations (Ting et al. 1987) also showed nocturnal CO<sub>2</sub> fixation (CAM) in some *Clusia* species. This kind of CO<sub>2</sub> uptake is an adaptation often found in plants from dry habitats, that allows the plants to close their stomata during the day and thus avoid elevated water losses. Like all members of the family Guttiferae, *Clusia* plants have latex in nearly all their tissues. Quantities and colour of the latex vary between species but also between different plant organs of one plant. It is today generally accepted that latex plays a role in the defense of lactiferous plants against herbivores and micro-organisms (cf. Farrell *et al.* 1991). This kind of attack is more common in the tropics than in extratropical regions and consequently the presence of latex is more common in tropical plants than elsewhere (Lewinson 1991). Comparisons between closely related plant groups (sister groups) also demonstrated that the groups with latex are generally richer in species than their sistergroups without latex (Farrell *et al.* 1991). The hypothesis that the possession of latex means an advantage in the "struggle of life" of plants was thus corroborated. The chemistry of latex in Guttiferae is not very well known. This is in part due to the fact that chemists often investigate chemical components of plants using whole organs so that it remains unclear where the

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components found occur more specifically within these organs. Furthermore, if plant material for chemical studies is used that is not really fresh or even dried major modifications in the chemistry of latex may lead to erroneous conclusions.

In *Clusia* as well as in other laticiferous (and resiniferous) plants it was sometimes observed that certain bees collect semi-dried latex exsuded from wounds of the plants (e.g., Armbruster 1984, Roubik 1989). The bees may even inflict minor wounds to provoke the exsudation of latex. These bees use the sticky, plastic semi-dried latex in the construction of their nest. It is obvious that this latex-collecting behaviour of bees is of no advantage for the plants. However, in a few plant groups, the attraction of certain bees to such a plastic, later hardening, waterproof substance led to the evolution of a new floral reward in the form of floral resin. As the great majority of *Clusia* is dioecious, i.e. male and female flowers are located on different plants, some agent is necessary to guarantee that pollen from male flowers reaches the stigma of the female flowers (= pollination). The resin, attracting bees in search of nest constructing material, serves as a reward for the bees, which by collecting floral resin on different plants, transport pollen to the stigmas. Besides in many *Clusia* species such floral resins up to now were found only in the genus *Clusiella* Planch. & Triana (Guttiferae), and in species of the genus *Dalechampia* L. (Euphorbiaceae, Armbruster 1984, Bittrich & Amaral 1997). It is unclear, why the latex is substituted by resin in the flowers. Possibly as the latter is less fluid it can be easier secreted onto a specific place within the flower and thus guaranteed that the bees act as pollinators during their resin collection activity. Some *Clusia* flowers not only secrete resin but additionally oils of different functions (Bittrich & Amaral 1997). In some species (e.g. *C. grandiflora* Splitg. and related species) the oil is secreted in droplets on the anther tips of the male flowers and acts as an accessory pollenkitt, i.e. it makes the dry, powdery pollen stickier so that it adheres better on the bees' body. In other species (e.g., *C. renggerioides* Planch. & Triana and related species) where the pollen in the male flowers is mixed with resin, either the oil makes the viscous resin more fluid or the pollen is directly suspended inside the oily phase. Both conditions guarantee a certain mobility of the pollen grains inside the resin. Without such a mobility the pollen grains would have no chance to reach the stigma papillae when left accidentally by a bee within a resin drop on the stigma. As the pollen can not germinate within the waterfree resin, such a contact is indispensable. In the same species, the stigma itself also secretes a kind of oil which by mixing with a resin drop on the stigma liquefies the resin which spreads over the whole stigma guaranteeing the necessary contact of pollen grains and stigma papillae. *Clusia* flowers, especially in the group of species with floral resin, show an enormous variability in their floral morphology, matched only by a few other angiosperm genera. In those *Clusia* species with flowers that do not offer resin but pollen or nectar for their pollinators, the flower morphology is much less variable. It was suggested that the special characteristics of resin as a floral reward is at least partly responsible for the astounding variability of resiniferous *Clusia* flowers (Bittrich & Amaral 1996b). Freshly secreted resins are very sticky and this characteristic creates a problem for the plants especially in the male flowers, as pollen grains glued to the resin is probably completely lost for pollination. There are different ways out of the problem. Most often flower morphology in one or the other way impedes or minimizes the contact of pollen with the resin. In many *Clusia* species as well as in *Dalechampia* and in *Clusiella* various examples for this solution of the problem can be found (Bittrich & Amaral 1996b, 1997). The other possibility,

only evolved in certain *Clusia* species, is to make the resin less sticky or more fluid so that pollen grains in it remain mobile and thus are not lost for pollination.

It is still not quite clear why exactly certain bees are collecting the floral resins. It is known that it serves as constructing material for the nests with certain favourable characteristics: being for some time plastic and thus easy to manage for the bees, it later hardens and forms a cement which is completely waterproof. On the other hand it may have certain chemical characteristics which are also advantageous for the bees, like fungicidal or bactericidal activities. The presence of such activities in floral resins, however, does not prove that the bees are actively searching for this activity nor that the plants produce such a resin as a more attractive reward for the bees. Possibly the plants use biosynthetic pathways for the resin synthesis they also use for the synthesis of latex components, i.e. because the plant produced chemical substances for their own protection against bacterias and fungi, the resin accidentally got these activities, too. Another question is, if the bees are able to distinguish between a resin with bacterial and/or fungicidal activities and another which has “only” the characteristics of a useful cement. Perhaps well-designed field experiments will give us one day the answer to the latter question.



Figure 1: Details of *Clusia* species, flowers , resins and pollinators. a) trees with flowers of *Clusia nemorosa* (male) b)bee collecting flower resins from *Clusia nemorosa* (male) c) *Clusia nemorosa* (hermaphrodite) d) bee collecting flower resins from *Clusia grandiflora* (female) e) *Clusia grandiflora* (female) f) *Clusia insignis* (male).

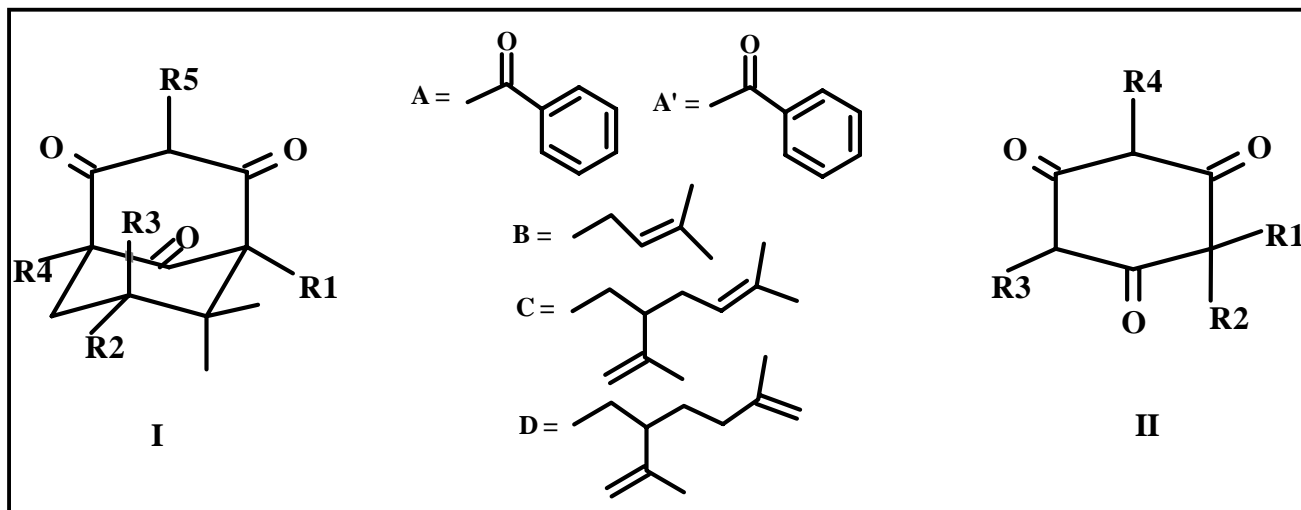
*Clusia* are not the only members of Guttiferae producing oily substances in their flowers (cf. Bittrich & Amaral 1996a). In the flowers of *Tovomitia* species, the stamens and staminodes secrete fragrant oils that attract male euglossine bees acting as pollinators (Nogueira *et al.* in press). In the flowers of *Symphonia* L. and related genera, the pollen is mixed with a floral oil, the same phenomenon was observed in *Garcinia gardneriana* (Planch & Triana) Zappi. It seems plausible that the ability to produce latex preadapted the plants for the production of floral oils

and resin, but only closer investigations of the chemistry of these substances can corroborate or reute this hypothesis.

## **CHEMICAL COMPOSITION OF THE CLUSIA FLORAL RESINS AND OILS**

Disclosing the chemical composition of *Clusia* floral resins and oils would bring to light many unanswered questions like: Do pollinating bees really use *Clusia* floral resins to build their nests? What is the chemical composition of these floral resins? Are these resins responsible for the chemical protection of the nest?

We have worked with the floral resins of 14 species of *Clusia* (table 1) belonging to 3 different sections (Oliveira, C M A *et al.*, 1996 and Oliveira, C M A *et al.* 1998). Fresh floral resins were collected and treated with diazomethane, and the methylated resins were submitted to a series of silica gel columns, thin layer and thin layer with silver nitrate chromatographies resulting in the isolation of ten pure compounds, nine of which were of which were novel polyisoprenylated benzophenone derivatives either possessing skeleton I or II (Figure 2). HPLC analysis revealed that polyisoprenylated benzophenones of type I or II are the major constituent of the resins. In some cases they constitute 70% of the resin. The other constituents are long chain carboxylic acids.



I						II				
#	R1	R2	R3	R4	R5	#	R1	R2	R3	R4
1	B	B	H	B	A	7	B	B	A	B
2	B	B	H	A	B	8	B	C	A	B
3	B	B	H	A'	B	9	B	D	A	B
4	A	B	H	B	B	10	C	C	A	B
5	B	H	B	A	B					
6	B	C	H	A	B					

Figure 2. Compounds isolated from the *Clusia* floral resins.

The chemistry of the floral oils was revealed by GC/MS analyses and they are mainly composed of long chain acids and esters. With the chemistry of *Clusia* floral resins and oils revealed it was possible to detect by HPLC clusianone **1**, a polyisoprenylated benzophenone, in the chemical composition of a *Trigona* bee nest. Thus confirming that the pollinating bees use the floral resins to build their nest ( at least *Trigona* bees).

Table 1: Quantification of compounds 1-10 present in <i>Clusia</i> floral resins by HPLC									
SPECIES OF CLUSIA	1	2	3	4	5	6	7	8+9	10
<b>C. BURCHELLII</b> <sup>1,6,8</sup>	55.4	-	-	-	-	2.7	-	22.2	-
<i>C. spiritu-sanctensis</i> <sup>1,6,8</sup>	76.8	-	-	-	-	1.0	-	-	-
<i>C. spiritu-sanctensis</i> <sup>2,6,8</sup>	78.6	-	-	-	-	16.9	-	-	-
<i>C. fluminensis</i> <sup>1,6,8</sup>	37.0	-	-	-	-	10.0	-	6.7	-
<i>C. hilariana</i> <sup>1,6,9</sup> (red)	-	-	-	-	-	-	-	11.3	-
<i>C. lanceolata</i> <sup>1,6,9</sup>	5.8	-	-	-	-	-	-	31.1	-
<i>C. panapanari</i> <sup>2,6,8</sup>	5.5	-	-	-	-	14.9	-	10.0	5.2
<i>C. paralicola</i> <sup>1,8,8</sup>	74.6	-	-	-	-	-	-	6.8	4.9
<i>C. pernambucensis</i> <sup>1,6,8</sup>	63.7	-	-	-	-	6.9	-	3.4	-
<i>C. renggerioides</i> <sup>1,4,6,8</sup>	6.7	-	-	10.5	43.5	-	-	-	-
<i>C. renggerioides</i> <sup>1,4,7,8</sup>	36.1	-	-	-	4.9	-	-	-	-
<i>C. renggerioides</i> <sup>1,5,7,8</sup>	-	-	-	-	17.0	-	-	-	-
<i>C. renggerioides</i> <sup>2,7,8</sup>	34.0	-	-	-	24.0	-	-	-	-
<i>C. weddelliana</i> <sup>1,6,8</sup>	56.5	-	-	-	-	6.5	-	20.0	7.0
<i>C. insignis</i> <sup>1,7,10</sup>	-	-	-	-	-	-	-	-	-
<i>C. nemorosa</i> <sup>1,6,10</sup>	-	11.8	12.0	-	47.6	-	-	-	-
<i>C. nemorosa</i> <sup>3,6,10</sup>	-	28.5	-	-	-	-	-	-	-
<i>C. grandiflora</i> <sup>1,6,10</sup>	-	15.1	-	-	-	-	6.0	-	-
<i>C. grandiflora</i> <sup>2,6,10</sup>	-	70.0	-	-	-	-	1.0	-	-
<i>C. rosea</i> <sup>2,6,10</sup>	-	33.0	-	33.0	-	-	-	-	-
nest	25.4	-	-	-	-	-	-	-	-

<sup>1</sup>male, <sup>2</sup>female, <sup>3</sup>hermaphrodite, <sup>4</sup>with pistilodium, <sup>5</sup>without pistilodium, <sup>6</sup>Instituto Agronômico de Campinas – IAC/SP, <sup>7</sup>Amazonas, <sup>8</sup>section *Cordylandra*, <sup>9</sup>section *Phloianthera*, <sup>10</sup>section *Chlamydoclusia*.

Biautography test revealed that the floral resin components had antimicrobial activity against *Bacillus subtilis* CCT 0089 and *Staphylococcus aureus* CCT 4295.

Finally isolation of 16 symbiotic fungi from *Trigona* bees allowed further insight in this ecosystem. As expected the fungi, belonging to genus *Aspergillus*, (*Demateaceos*, *Trichoderma*) were resistant to the benzophenone antimicrobial activity. On the other hand they showed a promising antibacterial activity.

## CONCLUSION

We are conscious that there are very few examples in the literature of three level interactions involving plant-pollinator-microorganism. We should point further that the perfect timing among botanist-chemists and microbiologist was responsible for this successful work.

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