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Article

Essential Oils of *Toona* and *Cedrela* Species (Meliaceae): Taxonomic and Ecological Implications

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Os óleos essenciais de *Toona ciliata*, *Cedrela odorata* e *C. fissilis* foram analisados por CG-EM. *Cedrela* apresentou em maior percentagem sesquiterpenos formados a partir do precursor pirofosfato de *cis*- e *trans*-farnesila. Já *Toona* mostra uma tendência em produzir principalmente sesquiterpenos derivados do pirofosfato de *trans*-farnesila. Estes resultados confirmam que a classificação destes dois gêneros em uma mesma tribo, Cedreleae, continua problemática. As respostas em eletroantenogramas médios (EAGs) dos óleos essenciais de *T. ciliata* e *C. odorata*, em fêmeas de *Hypsipyla grandella*, foram significativamente maiores que aquelas obtidas em machos, sugerindo que as fêmeas utilizariam os odores destes óleos para a seleção da planta hospedeira (gêneros de Swietenioidea) e na escolha de locais para oviposição.

The essential oils of *Toona ciliata*, *Cedrela odorata* and *C. fissilis* have been analysed by GC-MS. *Cedrela* contains the main sesquiterpenes formed from the *cis*- and *trans*-farnesyl pyrophosphate. In contrast, *Toona* tend to produce mainly sesquiterpenes formed from the *trans*-precursor. These results show that the affiliation of *Toona* in the tribe Cedreleae together with *Cedrela* is still rather problematic. Mean electroantennogram responses (EAGs) to the essential oils from *T. ciliata* and *C. odorata*, in *Hypsipyla grandella* females were significantly greater than those obtained for males, suggesting that females would use attractant odours messages from the host-plant (genera of Swietenioideae) as search strategy behaviour for habitat location and oviposition.

Keywords: Meliaceae, essential oil, chemotaxonomy, electroantennogram, gas chromatography-electroantennographic detection

Introduction

The family Meliaceae provides the most valuable timbers, such as mahogany (*Swietenia*) and cedar (*Cedrela*), which have been illegally exported from Brazil. At present they are scarce and efforts to establish large scale homogeneous plantations have almost invariably failed due to larval attacks by the shoot borer *Hypsipyla*. Main damage is caused by the larvae, which destroy the succulent terminal shoots by boring into the tip and tunnelling in the juvenile stems of saplings and seedlings. Re-sprouting of the plants, followed by repeated attacks of the insect, generally results in the development of numerous side branches and consequently in badly formed trees, unsuitable for timber production. *Hypsipyla grandella* is considered to be the most harmful species in Latin America, and *H. robusta* in Asia and Africa[1]. *Toona ciliata*, the Australian red cedar, introduced to Brazil shows excellent growth and an absence of attacks by *H. grandella*, in contrast to the native *Cedrela odorata*[2,3]. However, eggs of *H. grandella* have been found on the Australian cedar in field survey (Belém, Pará, Brazil).

Toona was originally described by Endlicher (1840) as a section of *Cedrela*. Later Roemer (1846) recognized that it could be separated by a number of sound morphological characters, raising *Toona* to generic rank[4]. Thus, the old world species of *Cedrela* were transferred to *Toona* (Endlicher) M. J. Roemer. The two genera were placed by Harms (1940) in the tribe Cedreleae under Cedreloideae[5]. Pennington and Styles (1975), in their more recent monograph, include Cedreleae into the Swietenioideae[6]. As reported in previous

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papers the known limonoids from *Cedrela* are typical of the Swietenioideae[7-9]. On the other hand, *Toona* differs from other genera of this subfamily, notably by the absence of limonoids of the mexicanolide group. Our earlier phytochemical studies on *T. ciliata*, showed the presence of limonoids with intact carbon skeleton 21-hydroxycedrelonelide, 23-hydroxycedrelonelide, 6α-acetoxy-14β,15β-epoxyazadirone and rings-A,C,D-intact-ring-B-*seco*-limonoids 12-deacetoxytoonacilin, 5α,6β,8α-trihydroxy-28-norisotoonafolin and 5α,6β,8α, 12α-tetrahydroxy-28-norisotoonafolin indicating that the genus is thus indeed akin to the Melioideae[10, 11]. Rings-A,C,D-intact-ring-B-*seco*-limonoids are features that are largely confined to the latter. We have now examined the essential oils of *T.ciliata*, *C.odorata* and *C. fissilis* in order to determine if the above differences still remain also in other classes of secondary metabolites. There is strong evidence that volatile principles of the host play a crucial role in the attraction of the females to oviposit. The electroantennograms (EAGs) were recorded from both sexes of *H. grandella* antennae to determine the stimulating capacity of the essential oils obtained.

Experimental

Isolation and analysis of volatile oil

Cedrela odorara and *Toona ciliata* were collected in Viçosa, MG, while *C. fissilis* in São Carlos, SP, Brazil; voucher specimens are deposited in the herbarium of Universidade Federal de Viçosa.

The fresh aerial parts of each species were submitted to steam distillation for 4 h, using a Clevenger apparatus. The essential oils obtained were dried over anhydrous Na_2SO_4 and kept in the freezer. The analyses of the oils were carried out on a Shimadzu GC-17A gas chromatograph fitted with a fused silica DB-5 (30 m x 0.25 mm ID, 0.25 mm film thickness) capillary column with helium as the carrier gas at a flow rate of 1.6 ml.min^{-1}. The temperature was programmed initially at 60ºC for 2min, then increased with a rate of 3ºC.min^{-1} to 240ºC. The injection was split and its temperature was 225ºC. The interface temperature was 250ºC. The chromatograph was coupled to a Shimadzu QP5000 mass selective detector at 70 eV; EIMS and CIMS (methane) for globulol (RI = 1583) and caryophyllene oxide (RI = 1581). Identification of the components was made by determination of their retention indices relative to those of an homologous series of *n*-alkanes[12], by comparison with a) co-injection with authentic samples, b) fragmentation patterns in mass spectra with those stored on the spectrometer database and bibliography[13].

Insects

Larvae of *Hypsipyla grandella* were collected in the fields of EIDAI, a Japanese timber company based in Belém, PA, Brazil. They were reared in a growth room at 26 ± 2ºC, 60±10 relative humidity and 16:8 hr light-dark photoperiod, at Universidade Federal de São Carlos, SP, Brazil. Pupae were separated by sex and in advanced age they were transported to Universidade Federal de Viçosa, MG, Brazil, where the electrophysiological analyses were conducted.

Electroantennograms (EAGs)

Antennae from 3-4-day-old males and females were used in the EAG (Syntech Laboratories) measurements. The antennae were cut off at the base. The tip of the terminal segment was removed to enable electric contact with the recording electrode. The tip and the base part were connected to an Ag-AgCl capillary electrodes filled with 0.1 N KCl with a small amount (5% by volume) of Polyvinylpyrrolidone (PVP).

Stimulus were delivered from glass odor cartridges (80mm long X 5mm ID) as 5 ml aliquots on Whatman no.1 filter paper pieces (7mm x 18mm). These odor cartridges were oriented towards the antenna and placed 1 cm away from the preparation. Odor molecules evaporating from the filter paper were carried over the preparation by humidifier air. Stimulus duration was 1 sec. Interstimulus time intervals of 1min. was allowed for recovery of the sensory cells. Control stimulations using air and filter paper impregnated with 10µl of hexane solvent were made at the beginning of each preparation. Maximal depolarization of the EAG during the stimulation period was used as a measure of antennal stimulation by the odorous stimulus.

The signal was amplified by a Data Acquisition Interface Board, Type IADC-02, developed by Syntech Laboratories and viewed on a EAG software package for WINDOWS.

Coupled gas chromatography-electroantennographic detection (GC-EAD)

The GC-EAD measurements were performed in a Shimadzu 17 A. GC with helium as carrier (50cm/s), equipped with an Supelcowax 10 column (0.25 film, 30m x 0.25 mm ID). Chromatographic parameters were as follows: initial oven 100ºC for 2 minutes, followed by a ramp of 5ºC/min. to a final temperature of 240ºC. Injections were done with split mode only. The sample was equally split between a flame ionization detector (FID) and the EAG detector. The EAG recorder, software, IADC (Intelligent Data Acquisition Controller) interface board for the AT486 PC and other peripheral equipament were also manufactured by Syntech Laboratories.

Experimental protocol

Two sets of experiments were performed in order to elucidate the selectivity of the antennal receptors of *H.*

grandella. In the first series of experiments, the general responsiveness of antennal receptors to individual oils (from *T. ciliata* and *C. odorata*) was measured by recording EAGs to volatile emanating from 10 µl stimulus load of each volatile. Four replicates were obtained for each sex. In the second series of experiments, one of the two most effective oil (leaves of *T. ciliata*) were tested. The GC-EAD equipment was used in order to identify some individual compounds in the oil mixture capable to elicit an EAG response. Two replicates were obtained only for females.

Statistical analyses

EAGs were compared statistically using analysis of mean and standard deviation. The EAG software calculates the values automatically.

Results and Discussion

Essential oils

The composition of the essential oils is given in Table 1. Component concentrations were calculated from GC peak areas and they were arranged in order of GC (DB-5) elution. Inspection of Table 1 clearly shows that all the oils consist largely of sesquiterpenes. The oils from the leaves (0.05%, V/W) and stems (0.05%, V/W) of *T. ciliata* contained 36 and 31 components, of which 96% and 92% were identified, respectively. The major compounds in both samples were β-caryophyllene, germacrene-D and bicyclogermacrene. Globulol was present in both oil, but in substantial amounts (12.50%) in the stems. Examination of the oils from the leaves (0.23%, V/W) and stems (0.03%, V/W) of *C. odorata* indicated the presence of 32 and 47 components, of which 95% and 78% were identified, respectively. The main constituents in the latter were β-caryophyllene and caryophyllene oxide, while in the former were β-elemene and germacrene-A. The volatile oils from both species examined seem to be characterized by accumulation of large amounts of β-caryophyllene. However, leaf oil of *C. odorata* contained higher amount of β-elemene, but very low β-caryophyllene.

The chemical composition of volatile oils of juvenile and adult leaves of *C. fissilis* were poorly differentiated (Table 1). The only exception refers to the main constituents. The juvenile leaf oil has a higher percentage of *cis*-4(14),5-muuroladiene than the adult leaf oil. The latter produces bicyclogermacrene as the principal compound.

Chemotaxonomic significance

A summary of sesquiterpene types from *T. ciliata*, *C. odorata* and *C. fissilis* are presented in Tables 2-5. For all species the percentages of each structural types were added (e.g. bicyclogermacrane: *T. ciliata*: 27.92 + 38.46 = 66.38%, Table 4). The skeletal types bicyclogermacrane, aromadendrane, aristolane, elemane, eudesmane, guaiane**,** germacrane and humulane can be assumed to derive from 2*E*,6-*E*-farnesyl pyrophosphate (*trans*, Figures 1-2), while the types seychellane, boubonane, cadinane, copaane, cubebane, longifolane, himachalane, longipinane, caryophyllane, bisabolane, acorane, chamigrane and cedrane derive from 2*Z*,6*E*-farnesyl pyrophosphate (*cis*, Figures 3-5). Intermediate cationic species have been invoked to explain how the various structures arise[14]. Each cationic intermediate can of course undergo rearrangement, or suffer hydride shifts, and functional groups may be introduced or modified, as some reasonable pathways are shown in Figures 1-5.

Toona has in common so many sesquiterpenes with *Cedrela* (Table 4). All oils are characterized by higher percentage composition of *trans*- than the *cis*-farnesyl derivatives. Both derivatives belong to 6 different skeletal types represented by 9 compounds, though with total yield of *trans*- and *cis*-farnesyl derivatives of 108.64% and 42.94% in *T. ciliata* and 109.29% and 52.67% in *C. odorata* and *C. fissilis* altogether, respectively. There are, however according to Tables 3 and 5, also fairly consistent differences between the sesquiterpenes of *Toona* and *Cedrela.* 4α-Methylaromadendranes **T.1.1**, **T.1.2** and **T.1.5** are known from the two latter, and **T.1.3**, **T.1.4** and **T.1.6** only from *T. ciliata*. The co-occurrence of bicyclogermacrane (**T.1**) and aromadendranes suggests that the biosynthesis of the latter compounds involves cyclization of a common bicyclogermacrane (**T.1**) precursor (Fig. 2). In this case, *T. ciliata* differs from *C. odorata* and *C. fissilis* by containing the enzyme systems necessary for both the cyclizations furnishing aromadendranes with a 4α- (**T.1.1** and **T.1.2**) and 4β-methyl (**T.1.3** and **T.1.4**) into five-membered ring.

In contrast, 4β-methyl-guaianes (**T.2.3**) occur in *C. odorata* and *T. ciliata*, whereas 4α-methyl-guaianes (**T.2.4**, **T.2.5** and **T.3.3**) are known only from *T. ciliata* (Fig. 1, Tables 3 and 4). All three species analysed develop cadinanes derived from the intermediates **C.1.2** and **C.1.1**, the cubebanes from **C.1.1** (**C.1.1.6**, Fig. 5) being restricted to *Toona*. Furthermore, *T. ciliata* contains representatives of aristolane (**T.1.7**), longifolane (**C.1.3.1**), bisabolane (**C.2.1**) and acorane (**C.2.2**), which do not seem to be common in *C. odorata* and *C. fissilis.* Copaane, longipinane, chamigrane, cedrane and eudesmane derivatives occur only in *Cedrela* (Table 5), but with highest concentrations in *C. odorata*, *C. fissilis* being limited to some cadinane (**C.1.2.3.1** and **C.1.1.1.2**) and longipinane (**C.1.3.3,** Table 1).

Table 1. Essential oil composition (%) from *Cedrela odorata*, *C. fissilis* and *Toona ciliata*

Components (codes)[a]	*Toona ciliata*		*Cedrela odorata*		*C. fissilis*		RI
	Leaves - adult (RI)	Stem (RI)	Leaves - adult (RI)	Stem (RI)	Leaves - adult (RI)	Leaves - juvenile (RI)	(Adams, 1995)
Sesquiterpene hydrocarbons	82.71	66.31	81.86	54.05	69.77	81.18	—
α-Cubebene (**C.1.2.4**)	—	—	—	—	7.89 (1360)	4.74 (1357)	1351
α-Ylangene (**C.1.1.3.1**)	—	—	0.69 (1370)	—	—	—	1372
α-Copaene (**C.1.1.2.2**)	—	—	2.02 (1374)	2.60 (1374)	—	—	1376
β-Bourbonene (**C.1.1.5**)	1.24 (1383)	—	0.90 (1384)	0.36 (1383)	—	—	1384
β-Cubebene (**C.1.2.4**)	0.21 (1388)	—	—	—	—	1.13 (1371)	1390
β-Elemene (**T.2.1**)	0.72 (1390)	0.65 (1390)	19.33 (1392)	5.40 (1390)	—	—	1391
β-Longipinene (**C.1.3.3**)	—	—	—	—	18.49 (1394)	1.55 (1396)	1398
Longifolene (**C.1.3.1**)	1.17 (1406)	—	—	—	—	—	1402
Z-Caryophyllene (**C.1.4**)	—	0.24 (1404)	0.84 (1404)	9.14 (1405)	—	—	1404
α-Gurjunene (**T.1.6**)	0.72 (1411)	3.40 (1408)	—	—	—	—	1409
β-Caryophyllene (**C.1.5**)	20.82 (1418)	10.04 (1417)	2.92 (1417)	17.18 (1417)	—	—	1418
β-Gurjunene (**T.1.7**)	0.35 (1427)	0.99 (1430)	—	—	—	—	1432
Aromadendrene (**T.1.1**)	0.58 (1437)	0.58 (1437)	1.17 (1438)	0.39 (1437)	—	—	1439
α-Guaiene (**T.2.3**)	—	—	2.28 (1442)	0.59 (1442)	—	—	1439
α-Himachalene (**C.1.3.2**)	—	—	1.16 (1448)	0.22 (1448)	—	—	1447
α-Humulene (**T.4.1**)	3.19 (1452)	1.24 (1452)	4.37 (1452)	7.65 (1452)	3.90 (1433)	0.58 (1431)	1454
Seychellene (**C.1.1.4**)	0.63 (1460)	1.61 (1459)	0.35 (1460)	0.30 (1459)	—	—	1460
Cis-4(14),5-muuroladiene (**C.1.2.3.1**)	—	—	—	10.45 (1459)	64.4 (1459)	1460	
γ-Gurjunene (**T.2.5**)	0.23 (1473)	—	—	—	—	—	1473
β-Chamigrene (**C.2.3**)	—	—	0.85 (1474)	0.21 (1475)	—	—	1475
γ-Himachalene (**C.1.3.2**)	0.37 (1477)	0.80 (1477)	1.14 (1477)	0.65 (1477)	—	—	1476
Germacrene-D (**T.3.2.1**)	16.36 (1481)	6.24 (1481)	1.39 (1480)	0.39 (1480)	—	—	1480
β-Selinene (**T.2.2**)	—	—	3.29 (1485)	1.56 (1485)	—	—	1485
Cis-β-Guaiene (**T.2.3**)	0.56 (1491)	—	—	—	—	—	1490
Bicyclogermacrene (**T.1**)	27.92 (1497)	38.46 (1497)	7.59 (1496)	—	26.62 (1474)	6.25 (1472)	1494
α-Selinene (**T.2.2**)	—	—	—	2.42 (1495)	—	—	1494
Epizonarene (**C.1.1.1.2**)	—	—	—	—	2.42 (1501)	2.53 (1500)	1497
α-Muurolene (**C.1.1.2.4**)	—	—	1.84 (1502)	0.34 (1501)	—	—	1499
Trans-β-Guaiene (**T.2.4**)	0.34 (1500)	—	—	—	—	—	1500
Germacrene-A (**T.3.1**)	0.98 (1505)	1.22 (1505)	22.56 (1506)	2.17 (1505)	—	—	1503
β-Bisabolene (**C.2.1**)	3.53 (1510)	—	—	—	—	—	1509
γ-Cadinene (**C.1.1.1.1**)	—	—	2.31 (1514)	0.45 (1514)	—	—	1513
7-epi-α-Selinene (**T.3.1.1**)	—	—	0.38 (1517)	—	—	—	1517
δ-Cadinene (**C.1.2.3**)	2.79 (1524)	0.84 (1524)	3.30 (1524)	2.03 (1524)	—	—	1524
Germacrene-B (**T.3.2**)	—	—	1.18 (1555)	—	—	—	1556
Oxygenated sesquiterpenes	13.21	25.85	10.04	26.95	—	—	—
Cubebol (**C.1.1.6**)	1.28 (1515)	—	—	—	—	—	1514
Elemol (**T.2.1**)	—	—	—	0.27 (1550)	—	—	1549
Ledol (**T.1.4**)	—	1.09 (1565)	—	—	—	—	1565
Spathulenol (**T.1.5**)	2.29 (1575)	3.17 (1575)	0.92 (1575)	1.73 (1575)	—	—	1576

Table 1. Cont.

Components (codes)[a]	*Toona ciliata*		*Cedrela odorata*		*C. fissilis*		RI (Adams, 1995)
	Leaves - adult (RI)	Stem (RI)	Leaves - adult (RI)	Stem (RI)	Leaves - adult (RI)	Leaves - juvenile (RI)	
Globulol (**T.1.3**)	4.16 (1581)	12.50 (1581)	—	—	—	—	1583
Caryophyllene oxide (**C.1.5.1**)	—	—	0.78 (1580)	17.85 (1580)	—	—	1583
Viridiflorol (**T.1.2**)	1.16 (1589)	3.32 (1589)	0.57 (1585)	—	—	—	1590
Guaiol (**T.3.3**)	—	0.88 (1591)	—	—	—	—	1595
Humulene epoxide II (**T.4.1.1**)	—	—	—	2.76 (1606)	—	—	1606
1-epi-Cubenol (**C.1.2.1**)	0.74 (1626)	—	0.84 (1627)	0.69 (1627)	—	—	1627
β-Acorenol (**C.2.2**)	—	2.37 (1636)	—	—	—	—	1634
epi-α-Muurolol (**C.1.1.2.3**)	—	—	—	1.18 (1641)	—	—	1641
Cubenol (**C.1.1.2.1**)	1.64 (1641)	1.19 (1641)	—	—	—	—	1642
α-Muurolol (**C.1.2.2**)	0.66 (1645)	—	—	—	—	—	1645
Selin-11-en-4-α-ol (**T.2.2**)	—	—	0.97 (1653)	2.47 (1653)	—	—	1652
α-Cadinol (**C.1.1.1.1**)	1.28 (1653)	1.33 (1653)	—	—	—	—	1653
Cedrane diol (**C.2.4**)	—	—	5.96 (1913)	—	—	—	1894
Diterpene							—
Cembrene A	—	—	6.45 (1917)	—	—	—	1942

[a]Codes: see Figs. 1-5 and Table 2.

Table 2. A summary of sesquiterpene types[a] classified in the biogenetic maps

Trans-farnesyl derivatives	*Cis*-farnesyl derivatives
Bicyclogermacrane (**T.1**)	Seychellane (**C.1.1.4**)
Aromadendrane (**T.1.1 – T.1.6**)[b]	Boubornane (**C.1.1.5**)
Aristolane (**T.1.7**)	Cadinane (**C.1.1.1.1 – C.1.1.1.2**)
Elemane (**T.2.1**)	Cadinane (**C.1.1.2.1 – C.1.1.2.4**)
Eudesmane (**T.2.2**)	Copaane (**C.1.1.2.2**)
Guaiane (T.2.3 – T.2.5)	Copaane (**C.1.1.3.1**)
Guaiane (**T.3.3**)	Cubebane (**C.1.1.6**)
Germacrane (**T.3.1 – T.3.2.1**)	Cubebane (**C.1.2.4**)
Eudesmane (**T.3.1.1**)	Cadinane (**C.1.2.1 – C.1.2.3.1**)
Humulane (**T.4.1 – T.4.1.1**)	Longifolane (**C.1.3.1**)
	Himachalane (**C.1.3.2**)
	Longipinane (**C.1.3.3**)
	Caryophyllane (**C.1.4 – C.1.5.1**)
	Bisabolane (**C.2.1**)
	Acorane (**C.2.2**)
	Chamigrane (**C.2.3**)
	Cedrane (**C.2.4**)

[a]The skeletal types are codified by letter and digits, which refer to the position of each skeletal type on the appropriate biogenetic map (Figures 1-5).
[b]The skeletal types (**T.1.1 – T.1.6**): from **T.1.1** to **T.1.6**: **T.1.1**, **T.1.2**, **T.1.3**, **T.1.4**, **T.1.5** and **T.1.6**.

Table 3. Sesquiterpene types identified only from *Toona ciliata*

Trans-farnesyl derivatives (7 / 24.66 %)[a]	*Cis*-farnesyl derivatives (6 / 11.84 %)[a]
Aromadendrane (3 / 21.87 %)[a]	Cadinane (2 / 3.49 %)[a]
T.1.3 (4.16 + 12.50)[b]	**C.1.1.2.1** (1.64 + 1.19)[b]
T.1.4 (1.09)	**C.1.2.2** (0.66)
T.1.6 (0.72 + 3.40)	**Cubebane (1 / 1.28 %)**
Aristolane (1 / 1.34 %)	**C.1.1.6** (1.28)
T.1.7 (0.35 + 0.99)	**Longifolane (1 / 1.17 %)**
Guaiane (3 / 1.45 %)	**C.1.3.1** (1.17)
T.2.4 (0.34)	**Bisabolane (1 / 3.53 %)**
T.2.5 (0.23)	**C.2.1** (3.53)
T.3.3 (0.88)	**Acorane (1 / 2.37%)**
	C.2.2 (2.37)

[a]Number of compound types and total percentage composition (21.87 + 1.34 + 1.45 = 24.66%)
[b]Percentage composition: see Table 1

The oils from *C. odorata* and *T. ciliata* were found to have markedly different chemical compositions to that identified by other workers. Representatives of cadinanes (cedrelanol; torreyol; α-muurolene; γ-muurolene; calamenene), one copaane (α-copaene), one cubebane (α-cubebene), one guaiane (guaiazulene) and two elemanes (β-elemene; δ-elemene) have been reported from the former[15-19]. Of these sequiterpenes only α-copaene and β-elemene were found in the present study. The second case is more interesting since the literature registers the occurrence of only α-copaene in *T. ciliata*[20]. The oils of *C. fissilis* had not been analysed previously.

Table 4. Sesquiterpene types identified from *Toona ciliata*, *Cedrela odorata* and *C. fissilis*[a]

Trans-farnesyl derivatives		*Cis*-farnesyl derivatives	
Toona ciliata (9 / 108.64)[b]	*Cedrela odorata* + *C. fissilis* (9 / 109.29)[b]	Toona ciliata (9 / 42.94)[b]	*Cedrela odorata* + *C. fissilis* (9 / 52.67)[b]
Bicyclogermacrane (1/66.38)[b]	(1 / 34.21)[b]	Cadinane (3/6.98)[b]	(3/9.62)[b]
T.1 (27.92 + 38.46)[c]	(7.59 + 26.62)[c]	**C.1.1.1.1** (1.28 + 1.33)[c]	(2.31 + 0.45)[c]
Aromadendrane (3/11.1)	(3/4.78)	**C.1.2.1** (0.74)	(0.84 + 0.69)
T.1.1 (0.58 + 0.58)	(1.17 + 0.39)	**C.1.2.3** (2.79 + 0.84)	(3.30 + 2.03)
T.1.2 (1.16 + 3.32)	(0.57)	Seychellane (1/2.24)	(1/0.65)
T.1.5 (2.29 + 3.17)	(0.92 + 1.73)	**C.1.1.4** (0.63 + 1.61)	(0.35 + 0.30)
Elemane (1/1.37)	(1/25.00)	Bourbonane (1/1.24)	(1/1.26)
T.2.1 (0.72 + 0.65)	(19.33 + 5.40 + 0.27)	**C.1.1.5** (1.24)	(0.90 + 0.36)
Guaiane (1/0.56)	(1/2.87)	Cubebane (1/0.21)	(1/7.89)
T.2.3 (0.56)	(2.28 + 0.59)	**C.1.2.4** (0.21)	(7.89)
Germacrane (2/24.8)	(2/26.51)	Himachalane (1/1.17)	(1/3.17)
T.3.1 (0.98 + 1.22)	(22.56 + 2.17)	**C.1.3.2** (0.37 + 0.80)	(1.16 + 1.14 + 0.22 + 0.65)
T.3.2.1 (16.36 + 6.24)	(1.39 + 0.39)	Caryophyllane (2/31.1)	(2/30.08)
Humulane (1/4.43)	(1/15.92)	**C.1.4** (0.24)	(0.84 + 9.14)
T.4.1 (3.19 + 1.24)	(4.37 + 7.65 + 3.90)	**C.1.5** (20.82 + 10.04)	(2.92 + 17.18)

[a]Chemotaxonomic studies refer only to adult leaves
[b]Number of compound types and total percentage composition
[c]Percentage composition: see Table 1

Table 5. Sesquiterpene types identified only from *Cedrela odorata* and *C. fissilis*[a]

Trans-farnesyl derivatives (4 / 15.03)[b]	*Cis*-farnesyl derivatives (10 / 65.68)[b]
Eudesmane (2/11.09)[b]	Copaane (2/5.31)[b]
T.2.2 (3.29 + 1.56 + 0.97 + 2.47 + 2.42)[c]	**C.1.1.2.2** (2.02 + 2.60)[c]
T.3.1.1 (0.38)	**C.1.1.3.1** (0.69)
Germacrane (1/1.18)	Cadinane (4/16.23)
T.3.2 (1.18)	**C.1.1.2.3** (1.18)
Humulane (1/2.76)	**C.1.1.2.4** (1.84 + 0.34)
T.4.1.1 (2.76)	C.1.2.3.1 **(10.45)**
	C.1.1.1.2 (2.42)
	Longipinane (1/18.49)
	C.1.3.3 (18.49)
	Caryophyllane (1/18.63)
	C.1.5.1 (0.78 + 17.85)
	Chamigrane (1/1.06)
	C.2.3 (0.85 + 0.21)
	Cedrane (1/5.96)
	C.2.4 (5.96)

[a]Chemotaxonomic studies refer only to adult leaves
[b]Number of compound types and total percentage composition
[c]Percentage composition: see Table 1

Based in the above evidences it is clear that *Cedrela* contains the main sesquiterpenes formed from the *cis*- and *trans*-farnesyl pyrophosphate [*trans*: 109.29% + 15.03% ; *cis*: 52.67% + 65.68% (Tables 4 and 5, respectively)]. In contrast, *Toona* tend to produce mainly sesquiterpenes formed from *trans*-precursor [*trans*: 108.64% + 24.66% ; *cis*: 42.94% + 11.84% (Tables 3 and 4, respectively)]. These interpretation of the sesquiterpene data and the fact that *Toona* differs from other genera of Swietenioideae[7-9], notably by the absence of limonoids of the mexicanolide group, are consistent with Roemer's[4] taxonomic conclusions, but suggested that the affiliation of *Toona* in the tribe Cedreleae together with *Cedrela* is still rather problematic.

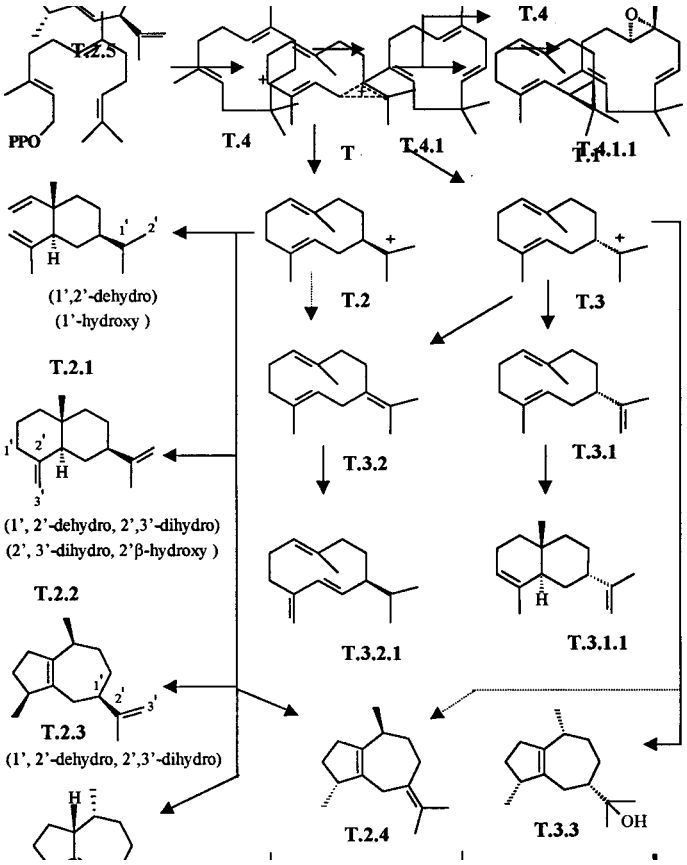

Figure 1. Part of a biogenetic map for sesquiterpenes featuring all structural types found in the oils from *Toona ciliata, Cedrela odorata* and *C. fissilis* (Tables 1-5). The codes (**1**, **2.1** etc.) refer to the position of the structural types on a map. The **T** letter indicates *trans*-farnesyl pyrophosphate as precursor.

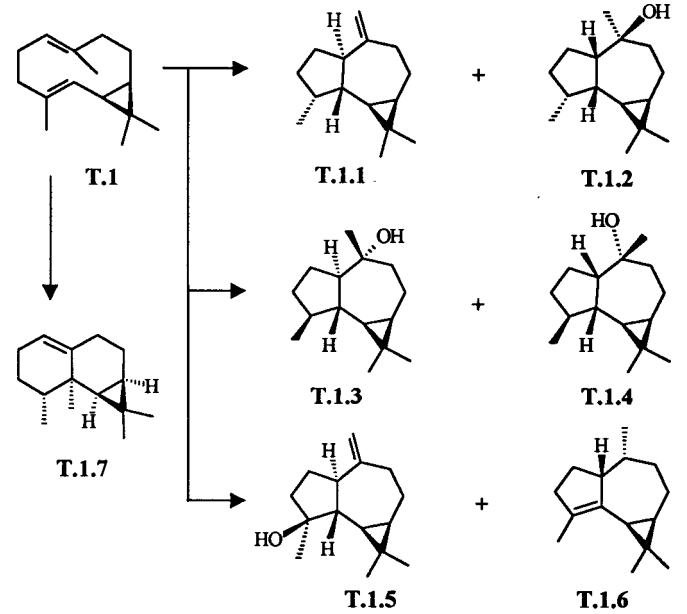

Figure 2. Part of a biogenetic map for sesquiterpenes featuring all structural types found in the oils from *Toona ciliata, Cedrela odorata* and *C. fissilis* (Tables 1-5). The codes (**1**, **1.1** etc.) refer to the position of the structural types on a map. The **T** letter indicates *trans*-farnesyl pyrophosphate as precursor.

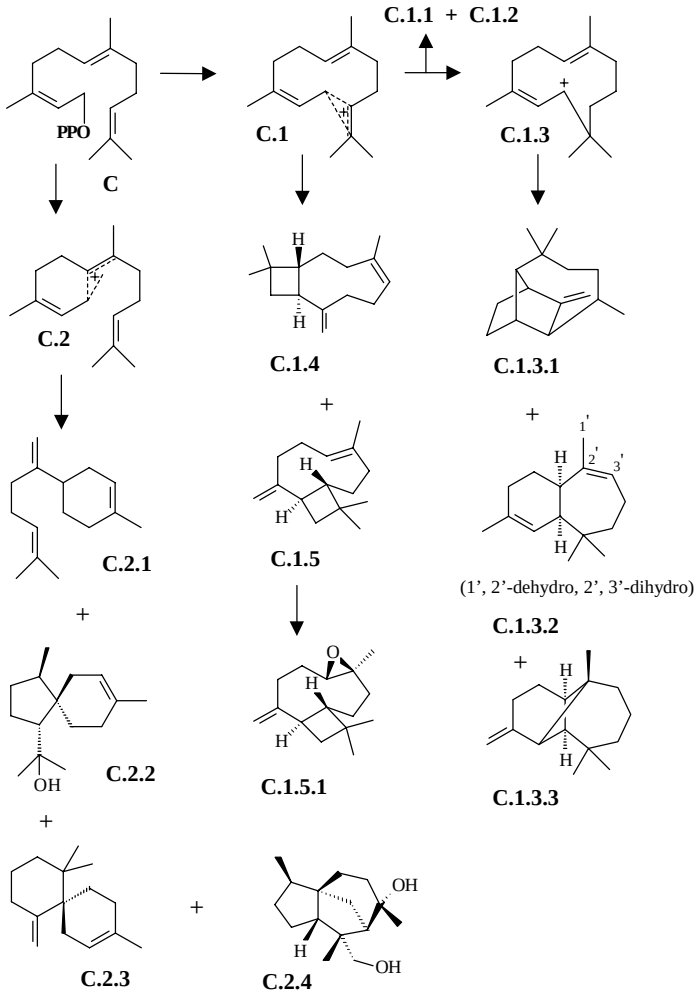

Figure 3. Part of a biogenetic map for sesquiterpenes featuring all structural types found in the oils from *Toona ciliata*, *Cedrela odorata* and *C. fissilis* (Tables 1-5). The codes (**1**, **1.3** etc.) refer to the position of the structural types on a map. The **C** letter indicates *cis*-farnesyl pyrophosphate as precursor. **C.1.1** and **C.1.2**: see Fig. 4.

Ecological significance

Mean electroantennogram responses (EAGs) to the essential oils from *T.ciliata* and *C. odorata* in *H. grandella* females, were significantly greater than those obtained for males (Figures 6 and 7). In addition, females were more selective to leaf oils than those from stems. The high selectivity of *H. grandella* female antenna to essential oils of *C. odorata* and *T. ciliata*, indicate its potential role as a chemical messengers for habitat location and oviposition (host-plant: genera of Swietenioideae[9]). Males, however, would not use similar information in its search behaviour, probably due to the fact that the relationship between males and the host-plant might not have the same importance. Furthermore, the results from EAGs to leaf oils from *T. ciliata* and *C. odorata* were not significantly different (e.g. Figure 6: -2.982 mV and -3.073 mV, respectively), indicating that adult females would be unable to detect the different volatile compounds of both oils. The main constituents in the former were β-caryophyllene (20.82%), germacrene-D (16.36%) and bicyclogermacrene (27.92%), while in the latter were β-elemene (19.33%) and germacrene-A (22.56%). They share only bicyclogermacrene as principal constituent (*T.c.* = 27.92%, *C.o.* = 7.59%). Attempts for locating potential components in leaf oils of *T. ciliata* by coupled GC-EAD failed (Figure 8). The absence of electrophysiological response might be related to an additive or synergistic effects of these compounds in the communication system of *H. grandella* female, which would not respond to an unique volatile, but to a mixture of two or more compounds. High EAG responses in previous expositions of the female antenna to the whole mixture confirm this theory (Figures 6-8). Field studies, optimal GC-EADs or/and EAG experiments with authentic samples are needed to clarify the role of essential oils in the *H. grandella* behaviour.

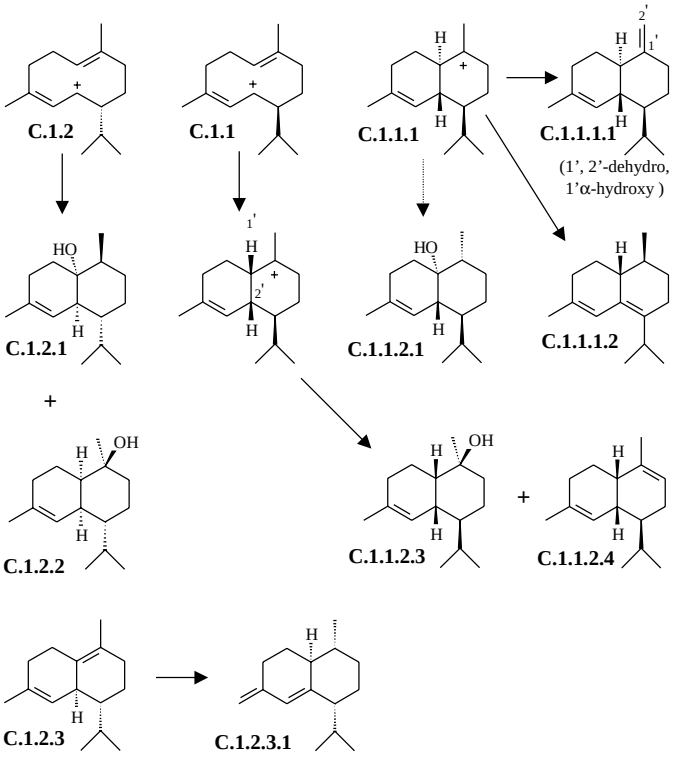

Figure 4. Part of a biogenetic map for sesquiterpenes featuring all structural types found in the oils from *Toona ciliata, Cedrela odorata* and *C. fissilis* (Tables 1-5). The codes (**1.1**, **1.1.1** etc.) refer to the position of the structural types on a map. The **C** letter indicates *cis*-farnesyl pyrophosphate as precursor.

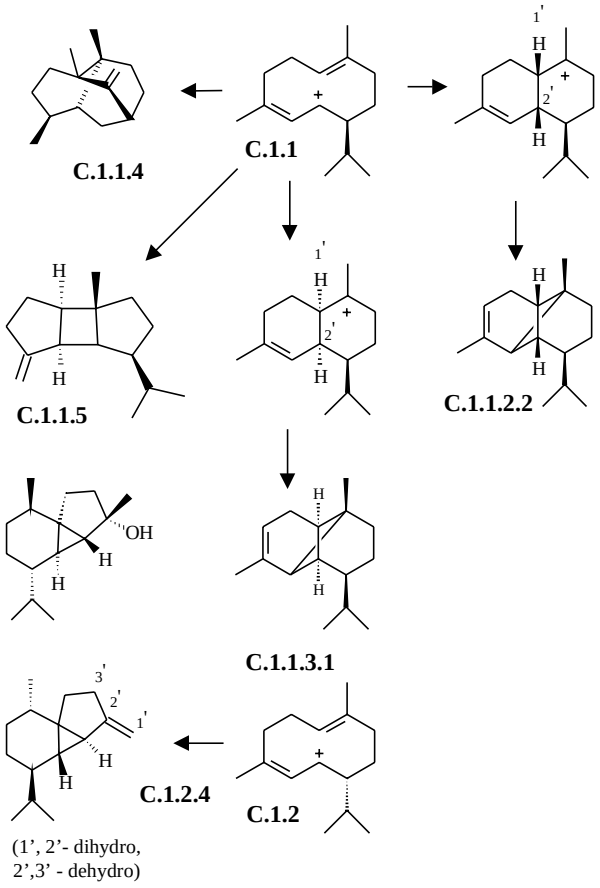

Figure 5. Part of a biogenetic map for sesquiterpenes featuring all structural types found in the oils from *Toona ciliata, Cedrela odorata* and *C. fissilis* (Tables 1-5). The codes (**1.1**, **1.1.4** etc.) refer to the position of the structural types on a map. The **C** letter indicates *cis*-farnesyl pyrophosphate as precursor.

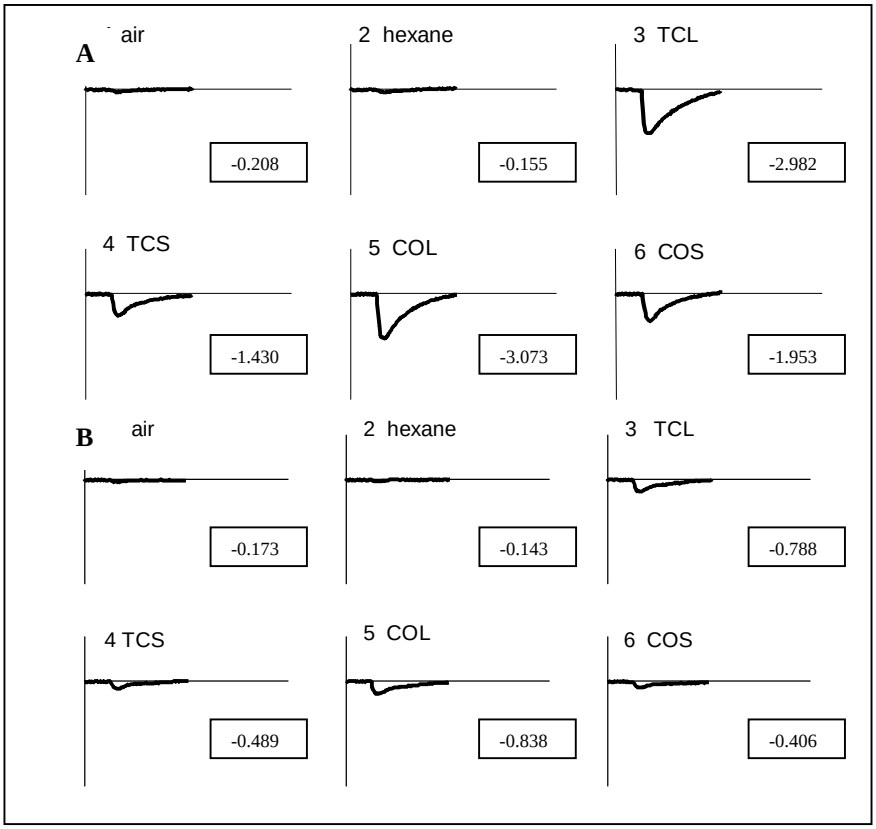

Figure 6. EAG responses of *Hypsiphyla grandella* females (A) and males (B) to essential oils from *Toona ciliata* (leaves: TCL, stems: TCS) and *Cedrela odorata* (leaves: COL, stems: COS) at 10µl stimulus load. Control: air and hexane. * Responses in mV.

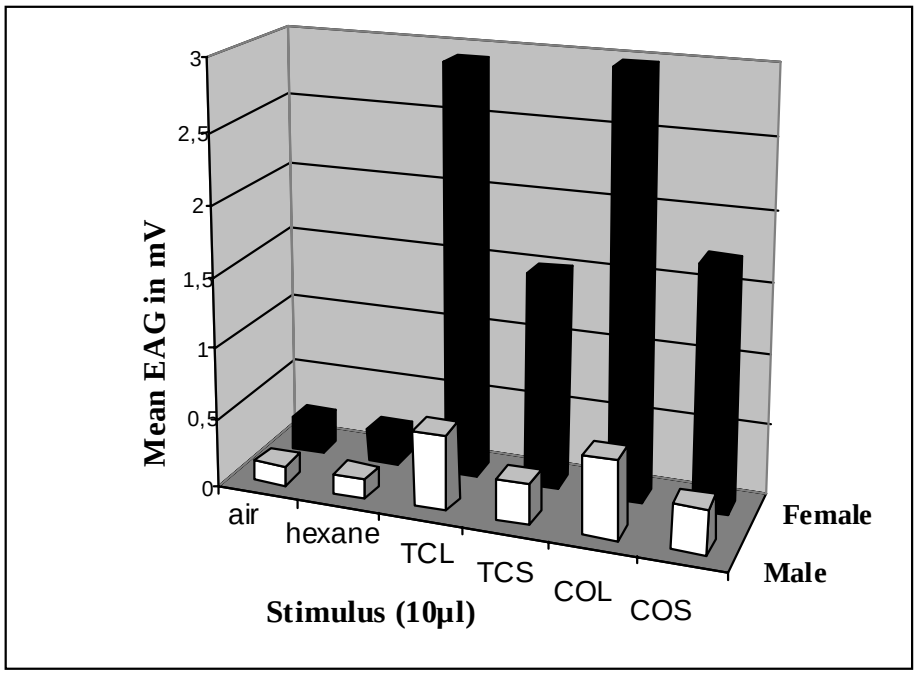

Figure 7. Mean EAG in millivolts elicited from *Hypsipyla grandella* males (□) and females (■) antennae, in response to essential oils from *Toona ciliata* (leaves: TCL, stems: TCS) and *Cedrela odorata* (leaves: COL, stems: COS) at 10µl stimulus load. Control: air and hexane.

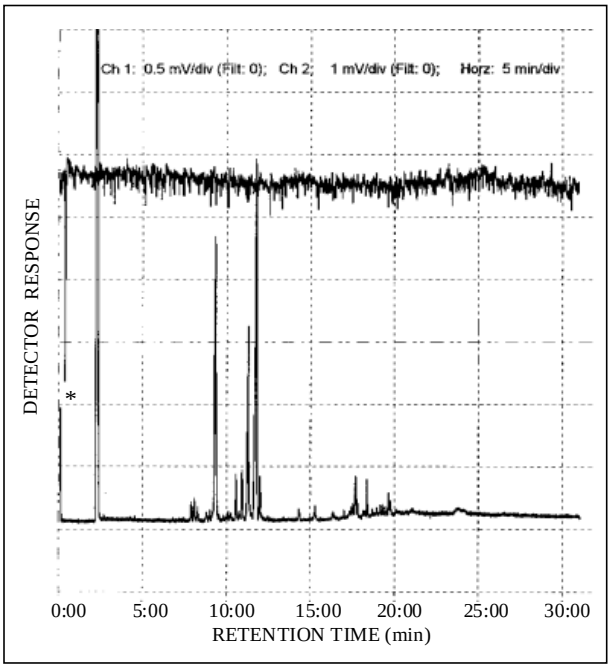

Figure 8. Simultaneously recorded EAD-FID chromatograms of *Hypsipyla grandella* female with FID responses to a essential oil (leaves) of *Toona ciliata*, injected under conditions described in the text. *Previous EAG response to the same oil tested prior to GC analysis.

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