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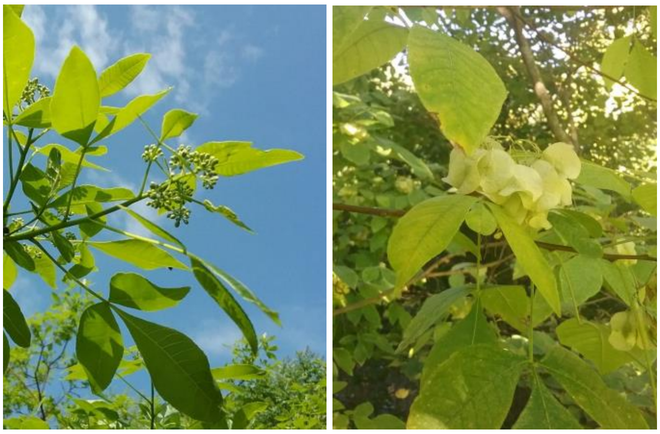
Essential oil compositions of male and female flowers of *Ptelea trifoliata*

William N Setzer and Prabodh Satyal

Abstract
The floral essential oils of *Ptelea trifoliata* were obtained from male and female trees by hydrodistillation and analyzed by gas chromatography – mass spectrometry. The male flowers were rich in germacrene D (13.8%), pentacosane (8.0%), (2*E*)-hexenal (7.7%), heptacosane (7.4%), α-humulene (6.1%), and tricosane (6.0%). The major components in the female floral essential oil were linalool (9.2%), myrcene (7.6%), heptacosane (7.1%), bicyclogermacrene (6.3%), pentacosane (6.1%), and germacrene D (5.7%). The essential oils showed qualitative similarities, but quantitative differences.

Keywords: floral essential oil, dioecious, gender differences, chemical composition, germacrene D, linalool, pentacosane, heptacosane, Rutaceae

1. Introduction
Ptelea trifoliata L. (hop tree, wafer ash, Rutaceae) is a dioecious shrub or small tree with trifoliate leaves, aromatic foliage, and small fragrant flowers (Figure 1) [1]. The roots and leaves have been used medicinally by Native Americans and early European settlers in North America, and the fruits have been used as a substitute for hops in beer brewing [2]. The essential oils from the leaves [1] and from the bark [3] have been characterized, but to our knowledge, the floral essential oils of *P. trifoliata* have not been previously examined. Floral volatiles are primarily used to attract pollinators, but floral secondary metabolites may also serve as defensive agents against herbivory and pathogenic microorganisms [4, 5]. Because *P. trifoliata* is a dioecious tree, the volatile chemistry of males and females may be different due to different resource allocation demands [6]. The purpose of this study is to analyze the floral essential oils of male and female *P. trifoliata* in order to discern differences, if any, in the compositions.



A B

Fig 1: *Ptelea trifoliata* L. **A:** Male tree budding. **B:** Female tree with fruits. Photographs by W.N. Setzer.

2. Materials and Methods
2.1 Plant Material
Male and female flowers were collected from individual trees (8 May 2019, 6:00 am) growing in Huntsville, Alabama (34°38′46.3″ N, 86°33′27.0″ W, elevation 192 m).

The plant was identified by W.N. Setzer and a voucher specimen (20190428-094721) has been deposited in the University of Alabama in Huntsville Herbarium. The fresh flowers (47.33 g male flowers and 17.08 g female flowers) were hydrodistilled using a Likens-Nickerson apparatus with continuous extraction with CH_2Cl_2 to give colorless essential oils (144.9 mg and 24.7 mg, respectively).

2.2 Gas Chromatographic–Mass Spectral Analysis

The floral essential oils of *P. trifoliata* were analyzed by GC-MS, as described previously [7], using a Shimadzu GC-MS-QP2010 Ultra fitted with a Phenomenex ZB-5ms column. Identification of the essential oil components was determined by comparison of their retention indices and their mass spectral fragmentation patters with those from the Adams [8], NIST17 [9], and FFNSC 3 [10] databases and our in-house library [11].

3. Results and Discussion

The flowers from the male *P. trifoliata* tree gave a clear colorless essential oil in 0.306% yield. A total of 88 compounds were identified in the floral essential oil, accounting for 99.3% of the composition. Female flowers, on the other hand, produced an essential oil in lower yield (0.145%) with 81 (98.6%) compounds identified. The chemical compositions of the two essential oils were qualitatively similar and showed only minor quantitative differences (Table 1). Knudsen and co-authors have found that 12 compounds occur in more than 50% of the floral scents examined [5]. Of these 12, six are found in both the male and female floral essential oil of *P. trifoliata*, namely limonene, (*E*)-β-ocimene, myrcene, linalool, α-pinene, and β-caryophyllene.

Table 1: Chemical compositions of male and female floral essential oils of *Ptelea trifoliata* L

RI[a]	Compound	Male%	Female%
800	Octane	---	0.3
802	Hexanal	0.4	0.8
849	**(2*E*)-Hexenal**[b]	**7.7**	**3.0**
853	(3*Z*)-Hexen-1-ol	---	1.2
862	**(2*E*)-Hexen-1-ol**	**0.2**	**4.0**
903	Heptanal	---	0.5
932	α-Pinene	0.4	1.5
971	Sabinene	0.5	1.3
988	**Myrcene**	**4.3**	**7.6**
1006	α-Phellandrene	0.1	0.3
1028	Limonene	0.3	0.7
1030	β-Phellandrene	2.0	3.7
1034	(*Z*)-β-Ocimene	tr[c]	---
1045	(*E*)-β-Ocimene	0.1	0.1
1070	*cis*-Sabinene hydrate	0.1	---
1099	**Linalool**	**4.7**	**9.2**
1104	Nonanal	0.4	2.0
1113	(*E*)-4,8-Dimethyl-1,3,7-nonatriene	---	0.1
1181	Terpinen-4-ol	tr	---
1187	Cryptone	tr	---
1195	α-Terpineol	0.3	tr
1206	Decanal	0.1	tr
1254	Chavicol	---	tr
1288	(*E*)-Anethole	---	tr
1292	2-Undecanone	0.3	tr
1309	*p*-Vinylguaiacol	0.4	0.1
1331	Bicycloelemene	0.2	0.2
1334	δ-Elemene	tr	tr
1340	α-Terpinyl acetate	---	tr
1342	5-Indanol	0.2	0.2
1348	Citronellyl acetate	0.5	0.1
1351	Eugenol	0.4	1.1
1374	α-Copaene	0.1	tr
1377	Geranyl acetate	0.1	tr
1382	β-Bourbonene	1.0	0.3
1385	α-Bourbonene	0.1	tr
1386	β-Cubebene	0.2	0.1
1388	β-Elemene	1.7	0.3
1392	(*Z*)-Jasmone	---	tr
1399	Methyleugenol	0.3	0.4
1406	Methyl *N*-methylanthranilate	---	tr
1417	β-Ylangene	0.4	---
1418	β-Caryophyllene	4.0	1.9
1428	γ-Elemene	0.4	0.1
1443	*iso*-Germacrene D	0.2	0.1
1447	(*E*)-*iso*-Eugenol	0.1	tr
1450	*cis*-Muurola-3,5-diene	0.1	tr
1451	(*E*)-β-Farnesene	tr	---

1454	α-Humulene	6.1	3.3
1467	Dauca-5,8-diene	0.1	tr
1474	γ-Muurolene	0.1	tr
1480	**Germacrene D**	**13.8**	**5.7**
1489	(2*Z*,6*E*)-α-Farnesene	2.1	---
1494	**Bicyclogermacrene**	**2.9**	**6.3**
1497	α-Muurolene	0.2	0.5
1502	(*E*,*E*)-α-Farnesene	3.0	0.3
1512	γ-Cadinene	0.1	0.2
1517	δ-Cadinene	0.5	1.1
1545	Elemicin	0.3	1.8
1548	α-Elemol	0.1	0.4
1557	Germacrene B	0.2	0.3
1560	(*E*)-Nerolidol	0.7	1.7
1576	Germacra-1(10),5-dien-4β-ol	0.8	1.8
1581	Caryophyllene oxide	0.1	0.2
1585	Globulol	0.1	0.2
1593	Methoxyeugenol	0.9	2.6
1609	Humulene epoxide II	0.1	0.1
1620	Germacra-1(10),5-dien-4α-ol	0.5	0.3
1642	τ-Cadinol	0.1	0.4
1644	τ-Muurolol	0.3	0.6
1646	α-Muurolol (= δ-Cadinol)	0.1	0.2
1655	α-Cadinol	0.8	1.8
1658	Selin-11-en-4α-ol	0.1	---
1737	Mint sulfide	0.1	---
1766	Benzyl benzoate	tr	0.3
1830	(2*Z*,6*E*)-Farnesyl acetate	0.1	---
1839	Phytone	0.1	---
1868	Benzyl salicylate	0.1	0.5
1958	Palmitic acid	0.6	---
1991	Ethyl palmitate	0.1	0.3
2000	Eicosane	tr	---
2020	(*E*,*E*)-Geranyl linalool	0.1	0.2
2094	Methyl linolenate	0.1	---
2096	Methyl oleate	0.2	0.3
2100	Heneicosane	0.1	0.2
2114	**(*E*)-Phytol**	**4.3**	---
2163	**Ethyl oleate**	**1.5**	**4.6**
2169	1-Docosene	---	0.5
2200	Docosane	0.2	0.2
2270	(9*Z*)-Tricosene	0.2	0.3
2300	Tricosane	6.0	4.8
2400	Tetracosane	1.4	0.8
2500	Pentacosane	8.0	6.1
2600	Hexacosane	0.8	0.6
2611	Auraptene	0.6	---
2630	Tetracosanal	0.1	---
2700	Heptacosane	7.4	7.1
2800	Octacosane	1.0	0.8
	Monoterpene hydrocarbons	**7.7**	**15.2**
	Oxygenated monoterpenoids	5.7	9.3
	Sesquiterpene hydrocarbons	**33.8**	**19.0**
	Oxygenated sesquiterpenoids	4.1	7.8
	Diterpenoids	4.4	0.2
	Benzenoids	2.4	6.8
	Fatty-acid derivatives	**11.9**	**17.5**
	Alkanes	24.8	20.9
	Others	0.9	0.3
	Total identified	95.7	97.0
	Number identified	88	81

[a]Retention Index determined with reference to a homologous series of *n*-alkanes on a ZB-5ms column.
[b] Notable concentrations differences between male and female compositions are highlighted in **blue bold**.
[c] tr = “trace” (< 0.05%).

It has been suggested that, for dioecious plants in general, resource allocation to reproduction in females leads to slower growth but greater investment in defensive secondary metabolites [12–14]. Differences in defensive secondary metabolites have included flavonoids, polyphenolics, and tannins [14], but there may also be effects in the production of essential oil yields and compositions [6]. A perusal of the literature has revealed some differences in essential oil compositions in male vs. female plants (Table 2).

Table 2: Variation in essential oil compositions of dioecious plants

Plant	Essential Oil	Comments	Ref.
Baccharis articulata (Lam.) Pers.	Aerial parts	Male EO yield = 0.16%; female EO yield = 0.15%. Male EO:>bicyclogermacrene (8.9% vs. 5.1%), > EO chemical diversity (45 components vs. 28 components).Female EO:> β-pinene (14.7% vs. 0.1%)	[15]
Baccharis caprariifolia DC.	Aerial parts	Male EO yield = 0.07%; female EO yield = 0.05%. Male EO: > α-terpineol (5.9% vs 0.0%), >spathulenol (8.4% vs. 4.5%), > α-cadinol (4.7% vs. 1.8%), > EO chemical diversity (23 components vs. 16 components). Female EO: > (*E*)-nerolidol (8.9% vs. 0.4%).	[16]
Baccharis dracunculifolia DC.	Aerial parts	Male EO yield = 0.19%; female EO yield = 0.18%. Female EO: > limonene (4.7% vs. 0.9%), > (*E*)-nerolidol (20.8% vs. 12.0%).	[16]
Baccharis erioclada DC.	Aerial parts	Male EO yield = 0.21%; female EO yield = 0.30%. Male EO:> β-caryophyllene (10.7% vs. 4.2%), >spathulenol (12.6% vs. 6.6%). Female EO: > α-pinene (8.5% vs. 0.3%), > β-pinene (21.4% vs. 1.2%), > limonene (15.2% vs. 2.7%).	[16]
Baccharis trimera (Less.) DC.	Flowered branches	Male EO yield = 0.20%; female EO yield = 0.18%. Male 69% sesquiterpene hydrocarbons, 15% oxygenated sesquiterpenoids; female 49% sesquiterpene hydrocarbons, 34% oxygenated sesquiterpenoids.	[6]
Cannabis sativa L.	Aerial parts	Exclusively in males: 1,8-cineole (5.8%), 3-methyl-3-cyclohexenone. Exclusively in females: two unidentified pyrazines (0.84% and 1.25%), two furanones (5,5-dimethyl-2(5*H*)-furanone and 5-*t*-butyl-2,2-dimethyl-3(2*H*)-furanone), 2-methylhexyl butanoate, 1,3,8-*p*-menthatriene, 2,4-dimethylanisole, sabinene, β-phellandrene.	[17]
Juniperus chinensis L.	Leaves	Male EO yield = 1.29%; female EO yield = 0.66%. Male EO: 40.6% oxygenated monoterpenoids, 14.8% oxygenated sesquiterpenoids. Female EO: 15.8% oxygenated monoterpenoids, 29.7% oxygenated sesquiterpenoids.	[18]
Juniperus communis subsp. *hemisphaerica* Nyman	Leaves	No major differences in yield or compositions.	[19]
Juniperus oblonga M. Bieb.	Leaves	Male EO yield = 0.60%; female EO yield = 0.30%. No major differences in EO compositions.	[19]
Juniperus scopulorum Sarg.	Branches	Male: > EO yield, > α-thujene, > sabinene, > myrcene, > limonene, > δ-cadinene. Female: > α-pinene, > α-terpinene, > γ-terpinene, > terpinolene, >pregeijerene B, >elemol, > 8α-acetoxyelemol.	[20]
Juniperus virginiana L.	Leaves	Male EO yield = 1.16%; female EO yield = 0.77%. No major differences in EO compositions.	[21]
Laurus nobilis L.	Aerial parts	Male EO yield = 1.8%; female EO yield = 0.6%. Male EO: > α-terpinyl acetate (12.0% vs. 5.1%). Female EO: > α-terpineol (7.3% vs. 1.8%).	[22]
Laurus nobilis L.	Flowers	Male EO: > 1,8-cineole (26.3% vs. 20.5%). Female EO: > (*E*)-β-ocimene (65.3% vs. 45.7%).	[22]
Magnolia kwangsiensis Figlar & Noot.	Flowers	No difference in EO yields. Male EO: > α-terpinene (13.0% vs. 7.1%), > α-amorphene (6.2% vs. 1.8%). Female EO: >*p*-menth-1-ene (5.9% vs 0.0%).	[23]
Pistaciaatlantica Desf.	Leaves	Male EOs: Myrcene 17.8-24.8%.Female EOs: Myrcene trace-1.3%.	[24]
Ptelea trifoliata L.	Leaves	Male EO yield = 0.10%; female EO yield = 0.24%. Male EO: 54.7% monoterpenoids, 45.3% sesquiterpenoids. Female EO: 30.8% monoterpenoids, 69.2% sesquiterpenoids.	[1]
Zanthoxylum alatum Roxb.	Leaves	Male EO: Linalool (10.0-35.6%), limonene (2.8-4.5%).Female EO: linalool (11.6-34.1%), limonene (1.6-6.5%).	[25]
Zanthoxylum clava-herculis L.	Leaves	No differences in EO yields. Female EO: > linalool (6.4-11.3% vs. 0.0-2.5%), > α-terpineol (2.7-3.5% vs. trace-1.6%); four compounds found in female but not in male (γ-muurolene, valencene, γ-cadinene, and δ-cadinene).	[26]

In this work, the floral essential oils of *Ptelea trifoliata* showed only minor differences in composition. The chemical diversity of the male flowers was slightly higher with 88 identified components compared to the female floral essential oil with 81 components. Monoterpenoids were slightly higher for the female essential oil (15.2% monoterpene hydrocarbons and 9.3% oxygenated monoterpenoids) than for the male (7.7% and 5.7%, respectively). Sesquiterpene hydrocarbons, on the other hand, were more abundant in the male flowers (37.4%) than in the female (20.6%). Notable differences in component concentrations were germacrene D (13.8% and 5.7% for male and female, respectively), (2*E*)-hexenal (7.7% and 3.0%, respectively), phytol (4.3% in male, not observed in female), and linalool (4.7% and 9.2%, respectively).

Ptelea trifoliata is dioecious and cannot self-pollinate [27]. Furthermore, unless male and female trees are in close proximity, wind pollination is unlikely. Rather, insect vectors are necessary for normal pollination in this species [28]. Typical pollinating insects of *P. trifoliata* include bees, wasps, flies, moths, and butterflies [27, 28]. The floral clusters of *P. trifoliata* emit a fragrance reminiscent of orange blossoms [2], presumably to attract pollinators.

4. Conclusions
The floral essential oils of *Ptelea trifoliata* showed only minor differences in composition between the sexes. The similarity in the floral essential oils of male and female *P. trifoliata* is consistent with visitation by pollinating insects to both sexes; that is, both male and female flowers need to attract the same insect visitors to achieve efficient pollination.

5. Acknowledgments
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6. Conflicts of Interest
The authors declare no conflicts of interest.

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