Floral Aromatics of *Ptelea*: Chemical Identification and Human Response

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ABSTRACT. Many members of the citrus family (Rutaceae) are valued for the aromatic compounds emitted by their flowers. *Ptelea* species are unusually cold-hardy members of the Rutaceae, but conflicting descriptions of the fragrance of their unisexual flowers may discourage the use of these trees. We analyzed floral volatiles and human response to these chemicals to test the hypothesis that the fragrance of staminate and pistillate flowers of these species differs. Gas chromatography and mass spectrometry showed that most volatile chemicals emitted by flowers of *Ptelea trifoliata* and *Ptelea crenulata* are monoterpenes, sesquiterpenes, and esters. Most volatiles were emitted from flowers of both sexes, but ethyl benzoate and estragole were emitted only from pistillate flowers. When concentrations of aromatics differed between sexes, they were higher for pistillate flowers, except for *cis*-3-hexenyl butanoate and an unidentified terpene. For *P. crenulata* and *P. trifoliata*, respectively, 81% and 77% of survey responses were from volunteers who liked the fragrance. Panelists most frequently described the scent of flowers of *P. crenulata* of both sexes with the words citrus, lime, and sweet. Panelists distinguished between pistillate and staminate flowers of *P. trifoliata*, describing the odor of pistillate flowers most frequently with the words damp-earthly, spicy, and sweet; staminate flowers were perceived as light, fresh, grassy, and pleasant. This work represents the first analysis of floral volatiles of *P. crenulata* and resolves conflicting prior reports regarding the floral fragrance of *P. trifoliata*. We conclude that differences among people rather than the sex of flowers account for conflicting prior reports of floral fragrance. The scents of flowers of *P. crenulata* and *P. trifoliata* appeal to most people and are horticultural assets of these trees.

Fragrance is a major horticultural feature of ornamental plants (Giovannini et al., 2021). For example, consumers of rose bushes (*Rosa*) rated fragrance as more important than any other attribute except disease resistance (Waliczek et al., 2018). Since the 18th century, the North American species, *Ptelea trifoliata* (Rutaceae), has been used in England and Scotland as an ornamental shrub; however, it is rarely planted in North America, perhaps, in part, due to confusion about the floral fragrance (Harvey, 1981; Lancaster, 1995). Some have reported a strong citrus aroma (Nokes, 2001; Wasson, 2001), and Knuth (1908) reported that male inflorescences have a stronger hyacinth-like scent than do females. In contrast, some claim the odor is unpleasant and have likened it with the scent of a bobcat (Elmore and Janish, 1976; Kurz, 1997). The foliage of *P. crenulata* is also described as ill-smelling (McMinn, 1989). Given these disparate reports, it is possible that humans differ in their perception of the aromatic chemicals emitted from flowers of *Ptelea*.

Although horticultural attention has focused on *P. trifoliata*, commonly known as hop tree or wafer ash, the genus includes two other species, *P. crenulata* and *Ptelea aptera* (Yang and Applequist, 2015). *P. trifoliata* comprises five subspecies that occur across much of North America from Ontario to Florida and as far west as Nebraska and Arizona (Bailey, 1962; Yang and Applequist, 2015). *P. crenulata* is endemic to California, and *P. aptera* to Baja California (Yang and Applequist, 2015). The leaves of *P. trifoliata* are eaten by larvae of giant swallowtail butterflies (*Papilio cresphontes*) and grasshoppers (*Sciotocerca emarginata*) (Scriber and Dowell, 1991; Sword and Dopman, 1999). The larvae of the moths *Agonopterix pteleae* and *Agonopterix costimacula* feed exclusively on *P. trifoliata* in Illinois (Harrison and Berenbaum, 2005). The flowers attract a variety of insect families as well (Ambrose et al., 1985; Stewart, 2021).

Fragrance frequently alerts insects to floral nectar or pollen rewards, and aromatics may trick insects by mimicking insect sex pheromones (Schiesti et al., 1999; Schiesti and Ayasse, 2001). Floral fragrance may be the most important way that a flower attracts bees, because odor is a much easier identifier for bees to learn than are color and shape (Menzel et al., 1974; Schmetter, 1972). The chemicals most responsible for the attraction of insects may not be the most abundant. For instance, p-anisaldehyde accounts for only 0.5% of the fragrance blend of *Cirsium arvense*, but is an important attractant for pollinators (Theis, 2006).

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Fig. 1. Principal component analysis (PCA) was used to give an overall summary of the variance between the floral sexes and locations using all the chemicals predicted. The data were expressed as a set of indices called principal components (PC), which each explain a portion of the total variance in the data set. PC1 and PC2 show aroma compound differences between pistillate and staminate flowers of *Ptelea trifoliata* from gas chromatography. Location (Ames and New York) is marked by color.

Phenolics and terpenes are major groups of secondary metabolites present in plant fragrances. Phenolic compounds arise from the shikimic acid pathway. Simple carbohydrates from glycolysis and the pentose phosphate pathway are converted into phenylalanine, tyrosine, and tryptophan, which, in turn, form all the phenolic compounds (Herrmann and Weaver, 1999). There are two main biosynthetic pathways that produce aromatic terpenoids in plants, the mevalonic acid pathway and the methylerythritol phosphate pathway (Lichtenthaler, 1998). Terpenes primarily act as herbivore deterrents, whereas different phenolic compounds can deter herbivores and pathogens, attract pollinators and fruit dispersers, and absorb ultraviolet radiation (Gershenzon and Croteau, 1992; Taiz and Zeiger, 2010).

P. trifoliata blooms in late May to June in Iowa, and *P. crenulata* blooms in April (Ambrose et al., 1985; Dirr, 1998). This dioecious species has small greenish-white pistillate and creamy-white staminate flowers that form rounded inflorescences (Ambrose et al., 1985). As Ambrose et al. (1985) describe *P. trifoliata*, flowers of both species have a corolla composed of five, slightly pointed petals that give the flower an open, shallow-bowl shape.

Pistillate and staminate flowers of dioecious species may have different scent profiles. Staminate flowers of the perennial herb *Silene latifolia* emit more fragrance than do its pistillate flowers (Waelti et al., 2009). Several compounds were present only in either the pistillate or staminate flowers of three dioecious figs: *Ficus fistulosa*, *Ficus fulva*, and *Ficus punctata* (Grison et al., 1999). Staminate and pistillate flowers of *Laurus nobilis* had mostly similar compounds, although in slightly different percentages; however, linalool and its oxides, which made up 7.1% of the profile, were emitted only from staminate flowers (Flamini et al., 2002).

Although showy flowers, cold hardiness, and advantages for native insects are important horticultural traits; further investigation of floral fragrance was necessary for evaluating the ornamental potential of *Ptelea*. We sought to determine if pistillate and staminate flowers of *P. crenulata* and *P. trifoliata* differ in the aromatic chemicals they emit. We hypothesized that such differences explain that both highly favorable and negative descriptions of the scent of these flowers have been reported. An alternative hypothesis is that the range of descriptions is due to variation in perception among people. To investigate fragrance composition and perception, chemical analyses with gas chromatography and mass spectrometry and two types of sensory panels were used. A descriptive panel showed how people describe the fragrance, and an affective test demonstrated preferences among human respondents.

Materials and Methods

Volatile analysis

SAMPLING. Inflorescences of *P. trifoliata* were collected from a wooded area of Ames, IA, and at Evangola State Park in Irving, NY (with permission), in May and June 2018 and 2019. At least three staminate and three pistillate plants were sampled in both states during both years. Inflorescences of *P. crenulata* were collected with permission from Black Diamond Mines Regional Preserve at Antioch, CA, in Apr. 2018. Sampled inflorescences ranged from those with flowers with buds beginning to open to flowers at anthesis, with no flowers that had begun to senesce. Samples were kept hydrated during transport to a laboratory for analysis within 24 h of collection.

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY. Samples of flowers and attached inflorescence pedicels (no leaves) weighing

Table 1. Principal component analysis (PCA) was used to give an overall summary of the variance between the floral sexes and locations using all the chemicals predicted. The data were expressed as a set of indices called principal components (PCs), which each explain a portion of the total variance in the data set. Cumulative variance explained by the first four PCs was used to assess differences in aroma profiles between floral sex and location of *Ptelea trifoliata*. Aromatic compounds, organized by chemical category, that contributed at least 4% to the first four PCs are included in the table. Because PC percentages less than 4% are not included, the total percent for each PC column is less than 100%.

Cumulative variance explained			PC1 (%)	PC2 (%)	PC3 (%)	PC4 (%)
Chemical category	Retention index	Compound	36.1	58.8	72.5	80.3
Benzenoid	1024	Benzene, 1-methoxy-4-methyl				7.1
Benzenoid	1195	Estragole		6.2		
Cyclic ketone	1395	(Z)-jasmone	5.2			
Ester - aliphatic	1004	(Z)-3-hexenyl acetate				5.0
Ester - aliphatic	1118	2-hexenyl, propanoate			6.6	
Ester - aliphatic	1161	2-hexenyl butanoate		8.1		
Ester - aliphatic	1184	cis-3-hexenyl butanoate				4.0
Ester - aliphatic	1325	cis-hex-3-en-1-yl 2-methylbut-2-enoate	4.5			
Ester - aliphatic	1218	cis-3-hexenyl-methylbutanoate				14.0
Ester - aliphatic	1245	trans-2-hexenyl-3-methylbutanoate			6.5	
Ester - aromatic	1171	Ethyl benzoate				10.5
Ester - aromatic	1193	Methyl salicylate			5.7	
Ester - aromatic	1341	Methyl 2-methoxybenzoate	4.7			
Phenylpropanoid	1285	trans-anethole			4.2	
Phenylpropanoid	1317	cis-anethole		4.1		
Phenylpropanoid	1358	Eugenol		4.0		
Phenylpropanoid	1401	Methyleugenol	5.3			
Terpene	1124	E-4,8-dimethylnona-1,3,7-triene		7.3		
Terpene	1392	Unidentified, MW 204		7.9		
Terpene - monoterpene	975	Sabinene		4.3		
Terpene - monoterpene	989	β -myrcene		6.6		
Terpene - monoterpene	1030	β -phellandrene		4.8		
Terpene - monoterpene	1038	trans- β -ocimene	4.1			
Terpene - monoterpene	1048	cis- β -ocimene			5.4	
Terpene - monoterpene	1099	Linalool		6.1		
Terpene - monoterpene	1134	2,6-dimethyl-1,3,5,7-octatetraene, E,E-			5.1	
Terpene - monoterpene	1142	1,3,8-p-menthatriene	4.0			
Terpene - sesquiterpene	1351	α -cubebene	5.4			
Terpene - sesquiterpene	1370	α -ylangene				15.6
Terpene - sesquiterpene	1384	β -bourbonene	3.9			
Terpene - sesquiterpene	1390	β -elemene	4.2			
Terpene - sesquiterpene	1420	Caryophyllene			6.4	4.2
Terpene - sesquiterpene	1428	γ -elemene	4.7			

(Continued on next page)

Table 1. (Continued)

			PC1 (%)	PC2 (%)	PC3 (%)	PC4 (%)
Cumulative variance explained			36.1	58.8	72.5	80.3
Terpene - sesquiterpene	1433	β -ylangene	4.5			
Terpene - sesquiterpene	1504	α -farnesene			5.7	
Terpene - sesquiterpene	1523	δ -cadinene			4.3	
	1092	Unidentified, MW 134				7.8
	1208	Unidentified	4.9			
	1211	Unidentified	4.9			
	1362	Unidentified				6.5
	1365	Unidentified	4.6			
	1415	Unidentified	4.7			

0.75 g were sealed in 100-mL glass bottles with polytetrafluoroethylene septa. Bottles were placed into a water bath for 50 min at 40 °C to enhance the release of volatiles into the headspace onto a solid phase microextraction (SPME) fiber [2 cm-50/30 μ m divinyl benzene/carboxen/polydimethylsiloxane (StableFlex; Supelco, Bellefonte, PA)]. Analyses of volatile compounds were conducted on a gas chromatograph (GC) equipped with a splitless injection port and flame ionization detector (Hewlett-Packard 6890; Agilent Technologies, Santa Clara, CA). Volatiles were thermally desorbed (220 °C) for 3 min via the GC injection port onto a fused-silica capillary column [30 m $\times$ 0.25 mm $\times$ 0.25 μ m film thickness (SPB-5, Supelco)]. The column pressure was set at 124.0 kPa with a helium flow rate of 1.9 mL $\cdot$ min $^{-1}$. The oven temperature was held at 30 °C for 5 min and increased to 200 °C at a rate of 5 °C $\cdot$ min $^{-1}$ and held for 10 min. Flow rates of detector gases were air at 400 mL $\cdot$ min $^{-1}$ and hydrogen at 30 mL $\cdot$ min $^{-1}$. Volatile compounds were predicted using Kovats retention indices from aroma databases and GC-mass spectrometry (MS) analysis (Acree and Arn, 2004; Flavor & Extract Manufacturers Association, 2021). Quantitation of the volatile compounds was based on peak areas, which are responses from the flame ionization detector. It is worth noting that the condition of the flowers analyzed with GC was different from the flowers in the sensory panels because the GC flowers were heated to a higher temperature in the water bath.

GC-MS was performed on a GC (7890B GC/5977A MS; Agilent Technologies) with a mass selective detector. The GC conditions were the same as for chromatographic analyses. Mass-spectrometer conditions were set as follows: electron ionization positive (EI+) polarity, source electron ionization energy at 70 eV, MS source temperature at 230 °C, quadrupole temperature at 150 °C, and scan range from m/z 41 to 400, at a frequency scanning cycle of six scans/s. Mass of spectra of the volatile compounds was compared with a spectral library (MassHunter version B.07.00.1413, Agilent Technologies).

Sensory analysis

After gaining approval from the institutional Human Subjects Review Committee of our Institutional Review Board in 2018 and 2019, volunteer panelists at least 18 years old were recruited to assess human response to floral fragrance of *P. trifoliata* and *P. crenulata*. Although demographic information about panelists was not recorded, they varied in age, gender, and race. Flowers

of the two species were collected in Iowa and California and transported to a laboratory, as described, for analysis of volatile chemicals.

DESCRIPTIVE TEST. The goal of this analysis was to generate a list of descriptive words human subjects would choose to use to describe the floral scents of *P. trifoliata* and *P. crenulata* (Cairncross and Sjöström, 1950; Stone et al., 1974). Separated by species and sex, five or six flowers from an inflorescence at anthesis and not showing signs of senescence were placed into a 200-mL glass jar surrounded with aluminum foil with the opening covered with cheese cloth and a plastic lid; this allowed panelists to smell the flowers at the proper time without seeing the flowers. Leaves and as much of the peduncle as possible were eliminated from samples to limit investigation to floral fragrance, not that of vegetative parts. Flowers equilibrated in the jars 30 min to 1 h before the session. Fifteen volunteers participated in the test with *P. crenulata* in Apr. 2018 and 12 in the test with *P. trifoliata* in June 2018. At the initial session, each panelist received two jars: one containing staminate flowers and one containing pistillate flowers. On cue, panelists uncapped the jar containing the staminate flowers, sniffed them, and wrote down words describing the fragrance. Panelists then shared their words with one another and had an opportunity to discuss which of their collective words conveyed the same meaning so that synonymous or near-duplicate words could be eliminated. Because panelists might perceive the fragrance differently, agreement was not the goal of the discussion. Rather, the goal was to develop a collection of words that represented the perceptions of all panelists. Then the process was repeated with the pistillate flower. Researchers did not suggest any vocabulary to panelists.

In a second session 1 or 2 d later, the same panelists (15 in Apr., 12 in June 2018) participated in a test session in individual sensory booths. Each panelist received the six sample jars prepared as before with letter labels in a random order. Two contained pistillate flowers, two staminate flowers, and two were empty as a control. Panelists were given a list of the vocabulary words they had agreed upon at the first session to describe the scent. For each jar, they were given 2 min to remove the plastic lid, sniff, and choose the vocabulary words that best described the fragrance.

AFFECTIVE TEST. An additional 119 panelists for *P. crenulata* and 111 for *P. trifoliata* were recruited to smell both pistillate and staminate flowers and give their opinion of the floral

Table 2. Aromatic compounds present in pistillate and staminate floral aroma of *Ptelea trifoliata*. Mean peak areas of pistillate and staminate profiles were compared with analysis of variance tests for individual chemicals.

Chemical category	Retention index	Compound	Fragrance description ^z	Pistillate peak area	Staminate peak area	Sex difference (P)
Benzenoid	1024	Benzene, 1-methoxy-4-methyl	Floral	36.21	46.63	0.8
Benzenoid	1195	Estragole	Anise, licorice	481.7	0	0.004**
Cyclic ketone	1395	(Z)-jasmone	Floral	48.19	26.82	0.007**
Ester - aliphatic	1004	(Z)-3-hexenyl acetate	Banana, candy, floral, green	36.85	16.98	0.3
Ester - aliphatic	1118	2-hexenyl, propanoate	Fruit	10.21	13.8	0.8
Ester - aliphatic	1161	2-hexenyl butanoate	Apple peel, citrus, fresh	69.14	33.9	0.08
Ester - aliphatic	1184	cis-3-hexenyl butanoate	Apple peel, citrus, fresh	107.33	747.49	0.03*
Ester - aliphatic	1218	cis-3-hexenyl-methylbutanoate	Fruit, apple, pear	30.62	8.63	0.004**
Ester - aliphatic	1245	trans-2-hexenyl-3-methylbutanoate	Fruit	14.25	16.85	0.5
Ester - aliphatic	1325	cis-hex-3-en-1-yl 2-methylbut-2-enoate	Herb, sweet	21.16	10.99	0.03*
Ester - aromatic	1171	Ethyl benzoate	Chamomile, celery, fat, floral, fruit	29	0	0.0002***
Ester - aromatic	1193	Methyl salicylate	Almond, caramel, wintergreen, sharp	909.16	978.44	0.9
Ester - aromatic	1341	Methyl 2-methoxybenzoate	Fruit	275.47	173.93	0.2
Phenylpropanoid	1285	trans-anethole	Anise	624.26	201.23	0.03*
Phenylpropanoid	1317	cis-anethole		74.23	32.2	0.04*
Phenylpropanoid	1358	Eugenol	Burnt, clove, spice	94.05	16.4	0.004**
Phenylpropanoid	1401	Methyleugenol	Clove, spice	91.95	28.55	0.02*
Terpene	1124	E-4,8-dimethylnona-1,3,7-triene		82.58	20.49	0.0003***
Terpene	1392	Unidentified, MW 204		0	17.48	<0.0001***
Terpene - monoterpen	975	Sabinene	Pepper, turpentine, wood	165.55	97.02	0.5
Terpene - monoterpen	989	β -myrcene	Balsamic, fruit, geranium, herb, must	512.97	113.07	0.007**
Terpene - monoterpen	1030	β -phellandrene		105.98	109.33	0.9
Terpene - monoterpen	1038	trans- β -ocimene	Floral	859.3	164.79	0.0008***
Terpene - monoterpen	1048	cis- β -ocimene	Floral	809.66	152.26	0.0004***
Terpene - monoterpen	1099	Linalool	Coriander, floral, lavender, lemon, rose	93.86	54.72	0.0004***
Terpene - monoterpen	1134	2,6-dimethyl-1,3,5,7-octatetraene, E,E-		373.23	74.13	0.0001***
Terpene - monoterpen	1142	1,3,8-p-menthatriene	Turpentine	61.55	17.35	0.002**
Terpene - sesquiterpen	1351	α -cubebene	Herb, wax	16.86	12.02	0.006**
Terpene - sesquiterpen	1370	α -ylangene		177.3	273.27	0.1
Terpene - sesquiterpen	1384	β -bourbonene	Herb	76.79	18.68	0.006**
Terpene - sesquiterpen	1390	β -elemene	Herb, wax, fresh	23.2	11.81	0.002**
Terpene - sesquiterpen	1420	Caryophyllene	Fried, spice, wood	24.1	13.63	0.007**
Terpene - sesquiterpen	1428	γ -elemene	Green, wood, oil	33.9	16.75	0.007**
Terpene - sesquiterpen	1433	β -ylangene		64.56	17.89	0.01**
Terpene - sesquiterpen	1441	Aromadendrene	Herb, fruit	182.44	124.57	0.1
Terpene - sesquiterpen	1443	Iso-germacrene D	Wood, spice	31.51	19.14	0.03*
Terpene - sesquiterpen	1453	α -humulene	Wood	59.94	26.54	0.03*
Terpene - sesquiterpen	1476	γ -Murolene	Herb, wood, spice	20	18.98	0.05*
Terpene - sesquiterpen	1481	Germacrene D	Wood, spice	18.77	14.1	0.002**
Terpene - sesquiterpen	1504	α -farnesene	Boiled vegetable, floral, wood	8.38	9.86	0.3

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Table 2. (Continued)

Chemical category	Retention index	Compound	Fragrance description ^z	Pistillate peak area	Staminate peak area	Sex difference (P)
Terpene - sesquiterpene	1523	δ-cadinene	Thyme, medicine, wood	10.44	12.55	0.5
	1092	Unidentified, MW 134		143.18	189.35	0.7
	1208	Unidentified		61.38	11.03	<0.0001***
	1211	Unidentified		28.33	14.84	<0.0001***
	1362	Unidentified		25.16	13.99	0.04*
	1365	Unidentified		17.54	15.99	0.02*
	1415	Unidentified		13.85	7.18	0.1
	1436	Unidentified		45.36	24.62	0.6
	1437	Unidentified		42.72	49.14	0.6
	1439	Unidentified		44.77	30.5	0.03*

^zFrom Acree and Arn (2004); Cuchet et al. (2019); Flavor & Extract Manufacturers Association (2021).* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

fragrance (Lim, 2011). Inflorescences, with their flowers mostly at anthesis, had all leaves removed to focus panelist attention on the floral fragrance. When testing *P. crenulata*, several inflorescences were used from one male tree and five female trees from California for the 3 d of testing. For *P. trifoliata*, inflorescences from six male and six female trees from Iowa were used for 3 d. Bouquets were placed at least 1 m apart to limit fragrance contamination between pistillate and staminate inflorescences. To prevent order-of-presentation bias, half the volunteers smelled pistillate flowers first and then staminate, and half did the reverse. Panelists indicated their separate preferences for pistillate and staminate flowers on a survey with a nine-point hedonic scale (1 = dislike extremely, 9 = like extremely). In addition, they were asked to explain their preferences by describing the fragrances.

Statistical analysis

For *P. trifoliata* only, differences between staminate and pistillate aromatic profiles were determined by analysis of variance (ANOVA) tests for individual chemicals and principal component analysis (PCA) with RStudio (version 4.0.3, car and factoextra packages; R Development Core Team, Vienna, Austria). PCA was used to give an overall summary of the variance between the sexes of the flowers using all the chemicals predicted. The data were expressed as a set of indices called principal components, which each explained a portion of the total variance in the data set. The numerous words generated by panelists during the descriptive assessments were categorized into groups based on similarities, and χ^2 Fisher's exact tests (JMP Pro version 14; SAS Institute, Cary, NC) were used to determine response differences between species and sexes. Affective test preferences were converted to a scale of numbers for statistical analysis, with extremely dislike coded as 1 and extremely like coded as 9. Scores were compared with ANOVA and Tukey's honestly significant difference test (JMP Pro version 14).

Results

Chemical analysis

PCA confirmed differences between sexes of *P. trifoliata* [$P < 0.001$ (Fig. 1)]. The first four principal components together explained 80% of the variance (Table 1). Almost all of the chemicals predicted contributed at least 4% to one of the first four principal components (Table 1). Although our purpose focused on differences between the sexes, our statistical analysis also accounted for differences in location (Fig. 1, Table 2). With the exception of *cis*-3-hexenyl butanoate and an unidentified terpene, all chemicals that differed between the sexes had higher peaks among the females (Table 2). Ethyl benzoate and estragole were present in female flowers only (Table 2).

Descriptive test

Volunteers developed vocabulary lists that differed by species ($P < 0.01$) and by sex for *P. trifoliata* only [$P < 0.0001$ (Tables 3 and 4)]. Volunteers easily distinguished empty jars from those with flowers ($P < 0.0001$). Words most frequently used to describe the fragrance of pistillate and staminate flowers of *P. crenulata* were citrus, sweet, lime, banana, mint, and pungent (Table 5). Pistillate flowers of *P. trifoliata* were most commonly described with the words damp-earthly, spicy, sweet, and citrus (Table 4). Staminate flowers of *P. trifoliata* were most frequently described as light, fresh, and grassy (Table 5).

Table 3. List of vocabulary that panelists used in the second session of the descriptive test to describe *Ptelea crenulata*. To simplify the panelists' descriptions for statistical comparison with a χ^2 Fisher's exact test, the panelists' words were grouped into categories listed at the top of each column.

Citrus	Clean chemical	Floral	Fruity	Other	Spices	Vegetative
Citronella	Fresh	Daffodil	Fruity	Dark	Allspice	Airy
Citrus	Latex	Dainty	Melon	Dull	Baked goods	Alfalfa
Citrusy-cleaning-supplies	Medicinal	Floral	Passion fruit	Heavy	Basil	Bark-like
Lemon grass	Menthol	Honey	Peaches	Light	Chai	Cardboard
Lime	Sterile	Honeysuckle	Overripe-banana	Musky	Cinnamon	Earthy
Orange	Toothpaste	Judd viburnum		Pleasant	Cologne	Elf-like
		Lilac		Sweet	Herbal	Fermented corn
		Lily		Unpleasant	Licorice	Fermenting, decaying, wet plants
		Lily of the valley		Urine	Masculine	Freshly cut wood
		Prairie flower		Warm	Warm mint	Leafy
		Sweet nectar		Yellow	Sweet	Mossy
		Tulip			Mint	Mowed grass
		Silky			Nutmeg	Musty
					Pungent	Oaky
					Sharp	Sawdust
					Spices	Stems-of-cut-roses-in-water
					Tea	Tropical
						Vegetation
						Wet
						Woody
						Woody

Affective test

For *P. crenulata* and *P. trifoliata*, respectively, 81.1% and 77.0% of survey responses were from volunteers who liked the floral fragrance (Fig. 2). Those who extremely liked the fragrance of *P. crenulata* described it as spring-like, sweet, fresh, floral, peppery, or summer-like. Volunteers extremely liked the fragrance of *P. trifoliata* because it was floral, lilac-like, jasmine-like, citrus, candy-like, sweet, or slightly musky. Those who disliked the fragrance of *P. crenulata* described it as bitter, earthy, sour, skunky, sickly-sweet-medicinal, stale, *Cannabis*-like, fermented, eucalyptus-like, overwhelmingly fruity, similar to marigold leaves, or similar to wilting *Matthiola incana*. The fragrance of *P. trifoliata* was disliked because it was stinky, weedy,

heavy, musky, urine-like, faintly manure-like, mustard-like, eucalyptus-like, or caustic.

ANOVA tests showed no difference in preference for pistillate or staminate flowers of either *P. crenulata* ($P < 0.36$) or *P. trifoliata* ($P < 0.68$). In the test for a preference for either pistillate or staminate flowers of *P. crenulata*, 27.7% of participants liked one and disliked or were indifferent to the other. Of those 33 participants, 54.5% preferred staminate flowers. With *P. trifoliata*, 26.1% liked one and disliked or were indifferent to the other. Of those 29 participants, 55.2% also preferred staminate flowers.

No difference between mean scores pooled across sex was observed for different days of the panel for *P. crenulata* ($P < 0.07$)

Table 4. List of vocabulary that panelists used in the second session of the descriptive test to describe *Ptelea trifoliata*. To simplify the panelists' descriptions for statistical comparison with a χ^2 Fisher's exact test, the panelists' words were grouped into categories listed at the top of each column.

Citrus	Clean chemical	Floral	Fruity	Other	Spices	Vegetative
Citrus	Fresh	Floral	Fruity	Sweet	Cedar	Damp-earthly
Lemon grass	Clean linen		Ripe banana	Pleasant	Basil	Dark leafy lettuce
	Mouthwash		Bubble gum	Light	Herbal	Prairie
			Almonds	Mellow	Pungent	Cut hay
			Pineapple	Delicate	Sharp	Grassy
					Masculine	Meadow
					Spicy	Tree leaves
					Black pepper	Woody
					Menthol	
					Minty	
					Cinnamon	

Table 5. Panelists' most frequently chosen descriptors from the second session of the descriptive panel for pistillate and staminate flowers of *Ptelea crenulata* and *Ptelea trifoliata*.

<i>P. crenulata</i>				<i>P. trifoliata</i>			
Pistillate	Frequency (%)	Staminate	Frequency (%)	Pistillate	Frequency (%)	Staminate	Frequency (%)
Citrus	9	Citrus	7.3	Damp-earthly	7.14	Light	7.41
Lime	7.5	Sweet	7.3	Spicy	7.14	Fresh	6.48
Sweet	6	Banana	5.7	Sweet	6.35	Grassy	6.48
Banana	4.5	Lime	4.9	Citrus	5.56	Pleasant	6.48
Mint	4.5	Pungent	4.1	Pleasant	5.56	Damp-earthly	5.56
Earthy	4.5	Heavy	4.1	Dark leafy lettuce	4.76	Floral	5.56
Floral	3.8	Musty	4.1	Fresh	4.76	Sweet	5.56
				Banana	4.76		

or *P. trifoliata* ($P < 0.15$), indicating that the floral aromas were consistent throughout the studies.

Discussion

The floral fragrance of *P. crenulata* and *P. trifoliata* was appealing to most panelists in the affective analysis, and reactions to pistillate and staminate flowers were similar. The differences identified by panelists in the descriptive analysis between the fragrances of pistillate and staminate flowers of *P. trifoliata* were consistent with the differences in peak heights observed in the GCs (Tables 2 and 5). The PCA that summarized most of the variance contributed to the volatile compounds also confirmed a difference between the sexes of *P. trifoliata* (Fig. 1, Table 1). The fragrances of individual chemicals predicted correspond closely to panelists' descriptions (Tables 2 and 5). Many of the compounds are described as floral, fruity, herbal, minty, and spicy; linalool is associated with citrus (Table 2). β -myrcene, which smells balsamic, fruity, geranium-like, herbal, and musty, may be responsible for the musky odor that panelists described, whereas 1,3,8-p-menthatriene, sabinene, and δ -cadinene, which smell like turpentine and medicine, might explain the medicinal descriptions (Tables 2–4). Pistillate and staminate flowers of *P. trifoliata* had nearly identical aromatic compounds, although only pistillate flowers emitted ethyl benzoate and estragole. When concentrations differed, they were higher in pistillate than

in staminate fragrance profiles, except in the case of *cis*-3-hexenyl butanoate and an unidentified terpene.

Eugenol, methyl salicylate, estragole, anethole, myrcene, ocimene, linalool, caryophyllene, bourbonene, and farnesene occur in many plants and were emitted from the flowers of *P. trifoliata* (Table 1) (Knudsen and Tollsten, 1993). The floral aromatic compounds of this species include several chemicals known from flowers of other members of the Rutaceae (Fadamiro, 2010; Loper, 1972; Renner, 1962). Linalool, myrcene, caryophyllene, and γ -Muurolene are components of the fragrance of *Zanthoxylum clava-herculis* (Fadamiro, 2010). Flowers of *Citrus depressa* also emitted linalool and myrcene, as well as ocimene (Loper, 1972). Linalool is a major component of the floral extract of *Citrus sinensis*, which also contains myrcene and ocimene (Grajales-Conesa et al., 2012). The floral extract of *Citrus limon* includes myrcene, *trans*-ocimene, caryophyllene, α -farnesene, and p-cubebene (Grajales-Conesa et al., 2012). Flowers of *Boronia megastigma* emit small concentrations of myrcene from the calyx and nectaries (MacTavish et al., 2000). Major components of the bud stage are caryophyllene and humulene, whereas concentrations of myrcene and ocimene are found in flowers at anthesis (MacTavish et al., 2000).

It is not surprising that similar compounds are shared among *P. trifoliata* and other Rutaceae because our panelists chose the word citrus to describe the floral aromas of both species of *Ptelea*, and the fragrance of *P. trifoliata* had previously been described as possessing a strong citrus aroma (Table 3) (Nokes, 2001; Wasson, 2001). Several of the compounds predicted in *P. trifoliata*, such as *E,E*-2,6-dimethyl-1,3,5,7-octatetraene, methyl salicylate, eugenol, methyleugenol, estragole, linalool, myrcene, *trans*- β -ocimene, and α -farnesene, are emitted from flowers of *Hyacinthus*, which may explain why Knuth (1908) compared *P. trifoliata* to hyacinths; however, these chemicals are either equal to or higher in pistillate flowers than in the staminate, whereas Knuth (1908) said the staminate flowers of *P. trifoliata* had the stronger scent (Table 1).

A minority of panelists disliked the fragrance of both species of *Ptelea*, which corresponds with the descriptions of Elmore and Janish (1976) and Kurz (1997) (Fig. 2). Two of the 15 members of the descriptive panel for *P. crenulata* used the word urine to describe the fragrance, which may be the same scent that Kurz (1997) called the bobcat scent (Table 3). The panelists from the descriptive study most frequently included the seemingly contrasting citrus, fruity, musty, and pungent in their description of *P. crenulata*; sweet, spicy, and damp-earthly in pistillate *P. trifoliata*; and light, fresh, floral, and damp-earthly in staminate *P. trifoliata*. These contrasting scents reported by our

Fig. 2. Distribution of affective panel responses to hedonic-scale survey for preference of fragrance of *Ptelea crenulata* and *Ptelea trifoliata*. Each volunteer filled out a survey for both pistillate and staminate flowers, which are combined in this figure. For *P. crenulata*, 119 panelists participated, and 111 volunteers participated in the panel for *P. trifoliata*.

panelists from the same flowers attest to the complexity of these fragrances. The aromatic chemical profiles of staminate and pistillate flowers of *P. trifoliata* differed; however, results from our affective panel indicate that differences in the ability of individual humans to discern these differences may be more important for human preference than are differences in chemical composition (Tables 2 and 5, Figs. 1 and 2).

Setzer and Satyal (2019) recently analyzed the composition of essential oil obtained from the flowers of *P. trifoliata* and reported many of the same components as found in our headspace analysis, including linalool, methyleugenol, caryophyllene, germacrene D, humulene, γ -murolene, β -myrcene, β -ocimene, β -bourbonene, anethole, ylangene, and α -farnesene. Notably, limonene, a monoterpene present in many Rutaceae and other flowering-plant families, is not in the floral aroma of *P. trifoliata* (Fadamiro, 2010; Knudsen and Tollsten, 1993; Loper, 1972; MacTavish et al., 2000). Limonene was also absent from the leaf essential oil of *P. trifoliata*, but present in its bark oil (Steinberg et al., 2017).

When concentration differences in the components of the fragrances of the staminate and pistillate flowers of *P. trifoliata* did occur, most chemicals had higher concentrations in pistillate flowers, although *cis*-3-hexenyl butanoate and an unidentified terpene were exceptions (Table 2). Only pistillate flowers emitted ethyl benzoate and estragole (Table 2). Sex-based differences in the aromatic profiles of flowers of other species have been reported. For example, the pistillate flowers of *Ficus fistulosa* emitted several compounds that were not emitted from staminate flowers; however, other fig species showed the opposite results (Grison et al., 1999). These findings contrast with those reported for *Silene latifolia*, which emits more fragrant aromatics from staminate than from pistillate flowers (Waelti et al., 2009). Also, linalool and its oxides were unique to staminate flowers of *L. nobilis*, making the staminate flowers more aromatically complex than the pistillate (Flamini et al., 2002).

Although no clear aromatic trend presents itself among dioecious species, perhaps the strategy for *P. trifoliata* is to entice pollinators with a more complex and stronger fragrance to its pistillate flowers that do not offer a pollen reward. Higher concentrations among most of the terpenes in the pistillate flowers may also function by adding extra protection for the female organs and especially their developing seeds (Gershenzon and Croteau, 1992).

Scent is a key attractant of pollinators, and the flowers of *P. trifoliata* are mostly visited by bees, wasps, and flies (Ambrose et al., 1985; Pichersky and Gershenzon, 2002). It might be possible to postulate which chemicals in the fragrance profile of *P. trifoliata* attract bees, wasps, and flies by identifying which chemicals they have in common with other flowers known to attract bees, wasps, and flies. The spicy-floral-scented, bee-pollinated *Cattleia labiata* contains mostly caryophyllene, as well as myrcene, ocimene, (E,E)-2,6-dimethyl-1,3,5,7-octatetraene, humulene, germacrene D, and jasmone; all of which are present in *P. trifoliata* (Table 1) (Kaiser, 1993). Bees from the tribe Meliponini are strongly attracted to floral extracts of *C. sinensis* and *C. limon*, which share aromatic compounds with *P. trifoliata* (Table 1) (Grajales-Conesa et al., 2012). *Dictamnus albus*, which has myrcene and limonene in its fragrance, is pollinated by insects from bee, fly, and wasp taxa (Renner, 1962; Sgolastra et al., 2016).

Genes linked to the expression of floral aromatics have been identified. In *Clarkia breweri*, genes have been isolated that code for enzymes that synthesize linalool, methyl eugenol, methyl isoeugenol, benzyl acetate, and methyl salicylate (Pichersky and Gershenzon, 2002). Scent formation is regulated in floral epidermal cells, especially in the petals (Pichersky and Gershenzon, 2002). In the case of *Antirrhinum majus*, the concentration of the enzyme that produces linalool is higher on the parts of the petal closest to the nectar source, suggesting linalool may guide insects to the nectar (Pichersky and Gershenzon, 2002).

In conclusion, this research provides the first analysis of floral aroma of *P. crenulata* and indicates that differences between staminate and pistillate flowers of *P. trifoliata* and differences in how humans perceive the volatiles emitted from these flowers, account for prior negative and positive descriptions. The complex and generally favorable floral fragrance of pistillate and staminate flowers of *P. crenulata* and *P. trifoliata* plays an ecological role by attracting pollinating insects and enhances the horticultural value of these trees. Both sexes and species can be recommended as ornamentals because of their elegant flowers, glossy leaves, distinctive samara fruits, cold hardiness, support for insects, and floral fragrance. Especially in the case of *P. trifoliata*, which has a wide distribution, further research could compare the effect of genetic variation among different regions on floral fragrance and human preference.

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