Characterization of Essential Oil of *Agastache* Species

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A collection of 19 accessions of *Agastache foeniculum* (anise hyssop), *A. rugosa* (catnip giant hyssop), *A. nepetoides* (Korean mint), and putative hybrids were analyzed for essential oil content and composition by gas chromatography (GC) and GC/mass spectroscopy. There was significant variation in oil content of the different lines of *Agastache* spp., ranging from 0.07 to 2.73 (percent volume/dry weight) for leaves and from 0.10 to 3.00 (percent volume/dry weight) for flowers. Variation in the essential oil composition was high among lines of *A. foeniculum* but low among lines of *A. rugosa*. Twenty-six compounds were identified in the essential oils, with methylchavicol being the major constituent (46.7–94.6%) in 14 lines of *A. foeniculum*, *A. rugosa*, and putative hybrids. In contrast, δ-cadinol was the major oil constituent (39.6%) in *A. nepetoides*. Additional oil constituents found in these species in concentrations above 1% include β-bourbonene, bornyl acetate, γ-cadinene, α-cadinol, δ-cadinene, α-camphene, β-caryophyllene, damascenone, β-ionone, isomenthone, α-limonene, linalool, methyleugenol, β-myrcene, *cis*-ocimene, 7-octen-4-ol, pulegone, and spathulenol.

INTRODUCTION

Anise hyssop (*Agastache foeniculum* [Pursh] Kuntze), native to the Great Plains of the United States and Canada; catnip giant hyssop (*A. nepetoides* [L.] Kuntze), native to eastern and central United States and Canada; and Korean mint (*A. rugosa* [Fisch. & C.A. Mey.] Kuntze), native to China, Korea, and Japan are perennial aromatic plants of the Lamiaceae family which are grown for their culinary and ornamental value. Plants of this genus have been used for flavoring beverages, as a spice in foods, and against colds (Gildemeister and Hoffman, 1961). *Agastache* has also shown promise as a honey plant (Mayer et al., 1982; Widrlechner, 1991).

A. foeniculum and *A. rugosa* have potentially useful volatile oils. Polak and Hixon (1945) analyzed the essential oil from *A. foeniculum* and reported the presence of methylchavicol, limonene, and pinene. The volatile oil from *A. rugosa* was shown to contain 83.5–96.3% methyleugenol with significant varietal differences (Fujita and Fujita, 1973). Zamureenko et al. (1980) analyzed the essential oil of *A. foeniculum* and reported methylchavicol (43.7%) and eugenol (43.7%) as the major constituents. Nykanen et al. (1989) analyzed the essential oil of a single plant of *A. foeniculum* from Canada and identified 39 constituents with methylchavicol (74.6%) as the major constituent. Charles et al. (1991) identified the major essential oil constituents in the leaves of *Agastache* spp. and also found that methylchavicol was generally the major constituent. This paper expands that initial study by Charles et al. (1991) and further describes both the major and minor essential oil constituents in both the leaves and flowers of *A. foeniculum*, *A. rugosa*, *A. nepetoides*, and putative hybrids between *A. rugosa* and *A. foeniculum*. The objective of this study was to determine the chemical diversity in essential oil composition within *Agastache* species to evaluate their potential use as a source of aroma chemicals.

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Table I. Essential Oil Content of *A. foeniculum*, *A. rugosa*, *A. nepetoides*, and a Hybrid

USDA accession no.	essential oil content, % vol/dry weight	
	leaves	flowers
A. foeniculum		
Ames 3064[a]	0.80	0.83
Ames 3481	0.07	0.63
Ames 4546	0.28	0.58
Ames 4550	0.36	0.20
Ames 4569	0.20	0.10
Ames 7611	0.91	1.80
Ames 7765	1.06	1.88
Ames 7872	2.45	3.00
Ames 7988	1.08	1.80
Ames 8001	0.82	1.21
A. rugosa		
Ames 4721	1.53	2.30
Ames 4992	2.73	–[b]
Ames 5018	2.12	–
Ames 7722	1.92	–
A. nepetoides		
Ames 4716	0.18	–
hybrid *A. rugosa* × *A. foeniculum*		
Ames 4721[c]	1.53	2.30
Ames 4992[c]	1.56	2.27
Ames 5018[c]	1.74	–
Ames 7722[c]	1.47	2.50
A. foeniculum commercial source	1.95	–

[a] Identification number of the North Central Regional Plant Introduction Station, Ames, IA. [b] Data not available. [c] Female parental line; male line is unknown.

MATERIALS AND METHODS

Plant Material. The USDA Plant Introduction collection of 15 lines of *A. foeniculum*, *A. rugosa*, *A. nepetoides*, and four populations of putative hybrids of *A. rugosa* × *A. foeniculum* were field-grown at the North Central Regional Plant Introduction Station, Ames, IA (Clarion loam soil), in 1989 in a completely randomized design consisting of two rows of 49 plants each. Putative hybrids were identified in the field using leaf and inflorescence morphology. Senechal (1990) used gel electrophoresis to confirm the hybrid parentage of a sample of the putative

0021-8561/91/1439-1946$02.50/0

Table II. Essential Oil Constituents in Leaves of *A. foeniculum* L.

essential oil constituent[a]	% of total oil for USDA Ames accession no.[b]									
	7988	7765	7872	3064	8001	7611	4550	3481	4546	4569
α-pinene	0.01	0.03		0.04	0.09	0.25	0.44	0.74		0.52
α-camphene	0.01	0.03		0.02	0.10	0.41	0.90	1.53		0.96
7-octen-4-ol	0.07	0.11		0.13	0.08	0.45				0.26
β-myrcene	0.04	0.09	0.23	0.11	1.13	0.64				1.67
α-limonene	0.25	0.44	2.53	0.44	4.88	5.80	0.91		0.10	2.47
cis-ocimene			0.25			1.00	0.07			
linalool	0.03	0.04		0.08	0.10		1.04	0.86	6.80	2.04
dihydroterpineol							0.96			0.82
camphene hydrate	0.04	0.05	0.38	0.05	0.06	0.34				0.53
isomenthone			0.14			0.17	0.88			1.12
methylchavicol	94.6	92.5	92.6	92.1	82.1	77.7	17.9	13.5	6.70	6.27
pulegone		0.08	0.27	0.19		0.30	0.40			0.31
bornyl acetate	0.35	0.37	0.19	0.39	1.14	2.54	18.7	28.9	1.44	18.1
β-bourbonene		0.12	0.33	1.38		2.83	0.82	0.82	7.13	1.71
methyleugenol	0.50			0.85		1.48				
β-caryophyllene	0.55	0.43	0.55	0.30	1.03	0.65	1.61	2.23	12.3	2.44
γ-cadinene	3.22	3.98	2.07	2.67		3.72	1.66	3.82	29.3	0.66
δ-cadinene				0.05		0.03				
spathulenol	0.09	0.41	0.13		2.18	0.12	45.6	33.0	10.5	49.5
α-cadinol									15.6	

[a] Listed in order of elution. [b] Listed from high to low methylchavicol.

Table III. Essential Oil Constituents in Leaves of *A. rugosa* and *A. nepetoides*

essential oil constituent[a]	% of total oil for USDA Ames accession no.				
	A. rugosa				*A. nepetoides*
	4992	4721	7722	5018	4716
α-pinene			0.01	0.01	
α-camphene					0.01
7-octen-4-ol	0.46	0.66	0.54	1.21	0.04
β-myrcene	0.11	0.23	0.30	0.55	
α-limonene	2.09	4.40	6.75	10.6	0.08
linalool	0.02	0.01	0.01	0.10	0.05
camphene hydrate	0.12	0.11	0.23	0.34	
isomenthone	0.04	0.04	0.59	8.50	
methylchavicol	93.7	92.3	80.2	55.9	6.52
pulegone	0.06		1.84	8.72	
bornyl acetate					0.07
eugenol	0.36	0.09	0.06	0.15	
damascenone					4.43
β-bourbonene	0.08		0.01	0.11	2.55
methyleugenol		0.09	0.11	9.08	
β-caryophyllene	1.91	0.77	0.85	1.51	16.5
β-ionone					1.32
germacrene B	0.07	0.08	0.05	0.08	0.66
γ-cadinene	0.06	0.09	0.63	0.60	
δ-cadinene	0.01	0.13	0.06	0.19	
spathulenol	0.17	0.34	0.14	0.42	17.4
caryophyllene oxide					0.76
δ-cadinol					39.6
α-cadinol	0.41	0.31	0.27	0.68	4.54

[a] Listed in order of elution.

Table IV. Essential Oil Constituents in Leaves of *A. rugosa* × *A. foeniculum* Putative Hybrids

essential oil constituent[a]	% of total oil for USDA Ames accession no.			
	7722[b]	4721[b]	4992[b]	5018[b]
α-pinene		0.06	0.11	0.20
α-camphene			0.11	0.16
7-octen-4-ol	0.54	0.84	0.57	1.09
β-myrcene	0.07	0.78	0.58	1.30
α-limonene	2.85	7.85	13.8	14.4
cis-ocimene		0.62	1.46	2.95
linalool			0.07	0.12
camphene hydrate	0.14	0.19	0.13	0.30
isomenthone	0.55	0.31	3.06	3.33
methylchavicol	92.8	84.6	71.1	46.7
pulegone		0.18	0.47	4.51
bornyl acetate		0.40	0.45	0.49
eugenol		0.03	0.06	0.30
β-bourbonene	0.32	0.16	0.51	
methyleugenol	1.74	0.09		15.2
β-caryophyllene	0.42	0.72	1.16	1.65
germacrene B				0.12
γ-cadinene	0.37	0.08	3.85	3.25
δ-cadinene		1.48	0.81	0.21
spathulenol		0.04	0.34	0.63
α-cadinol				0.92

[a] Listed in order of elution. [b] Female parental line; male line is unknown.

hybrids. Fifty individual plants from each accession were harvested at the bloom stage, bulked together to reflect the population of each line, and dried at 35 °C. Essential oils were separately extracted from known weights of leaves and flowers.

Essential Oil Extraction. Essential oil was extracted by hydrodistillation. Dried plant tissue was thoroughly mixed, subsampled, and placed in a 2-L round-bottom flask with distilled, deionized water (1000 mL for 75 g of dry material), and the essential oil was extracted using a modified Clevenger trap (ASTA, 1968). For smaller plant samples, the amount of water was adjusted proportionally (1 g of dry material:13.3 mL of water). The distillation period was 1 h and 15 min, and the essential oil content was determined on an oil volume to tissue weight basis (Charles and Simon, 1990). Essential oil samples were stored in silica vials with Teflon-sealed caps at 2 °C in the dark.

Essential Oil Identification and Quantification. Essential oil samples were analyzed by gas chromatography (GC) using a Varian 3700 gas chromatograph equipped with FID and a Varian 4270 electronic integrator (Varian, Walnut Creek, CA). A fused silica capillary column (12 m × 0.22 mm i.d.) with an OV101 [Varian, poly(dimethylsiloxane)] bonded phase was used to separate the oil constituents. Direct injection of 0.5-μL oil samples with helium as the carrier gas (100:1 split–vent ratio) and oven temperature held isothermal at 80 °C for 2 min and then programmed to increase at 3 °C/min to 200 °C gave complete elution of all peaks (sensitivity 10^{-10}, attenuation 16). The injector and detector temperatures were 200 and 300 °C, respectively. Essential oil constituents were identified on the basis of retention time and by co-injection with standard compounds. The relative peak area for individual constituents was determined by a Varian 4270 integrator (Charles et al., 1990).

GC/Mass Spectrometry Analysis. Pure compounds [α-camphene, α-pinene, α-limonene, β-myrcene, bornyl acetate, *cis*-ocimene, eugenol, linalool, menthone, methylchavicol, methyleugenol, and pulegone (J. Manheimer)] and essential oil constituents were verified by GC/MS. A Finnigan (San Jose, CA) GC (9610) and MS (4000) hooked on-line to a Data General Nova/4 data processing system used electron impact analysis as previously described (Charles and Simon, 1990; Charles et al., 1990).

Tentative identification of sesquiterpenes was accomplished by matching the mass spectra of each compound with different

Table V. Comparative Analysis of Flower Oil Composition of *A. foeniculum* and *A. rugosa* × *A. foeniculum*

USDA accession no.	% of total oil of major essential oil constituent[a]						
	β-myrcene	α-limonene	methylchavicol	bornyl acetate	β-caryophyllene	γ-cadinene	spathulenol
A. rugosa × *A. foeniculum*							
Ames 4721[b]	1.27	8.58	85.2	0.10	0.60	0.98	0.05
Ames 4992[b]	1.04	24.7	51.0	1.08	1.38	3.01	0.34
Ames 7722[b]		3.28	76.3	12.9		4.87	
A. foeniculum							
Ames 3064	0.07	0.20	93.2	0.48	0.88	1.78	0.81
Ames 3481	0.44	1.50	64.4	14.3	0.53	5.12	4.20
Ames 4546			38.4	10.9	3.23	6.23	7.87
Ames 4550	0.81	2.88	49.9	8.84	2.24	4.91	14.6
Ames 4569	2.37	1.20	21.3	13.0	3.66	1.96	29.4
Ames 7611	1.22	2.55	86.8	1.21	1.51	2.07	1.24
Ames 7765	0.01	0.02	95.5	0.35	0.42	2.11	0.15
Ames 7872	0.14	1.69	94.8	0.14	0.45	1.13	0.04
Ames 7988	0.03	0.17	95.6	0.30	0.35	1.93	0.11
Ames 8001	1.11	3.92	88.7	0.77	0.74	1.66	0.40

[a] Listed in order of elution. [b] Female parental line; male line is unknown.

MS compound libraries for best fit (Finnigan; Heller and Milne, 1978; Stenhagen et al., 1974; Adams, 1989).

RESULTS AND DISCUSSION

The analysis of a collection of 19 accessions of *Agastache* spp. for essential oil content and composition is presented in Tables I–IV. The oil content of *A foeniculum* ranged from 0.07% to 2.45% for leaves and from 0.10% to 3.00% for flowers; from 1.53% to 2.73% for leaves and 2.30% for flowers of *A. rugosa*; from 1.47% to 1.74% for leaves and from 2.27% to 2.50% for flowers of putative hybrids of *A. rugosa* × *A. foeniculum* (Table I). The oil content of the leaves of *A. nepetoides* was low (0.18%) as compared to that of a commercial source of *A. foeniculum* (1.95%). Diversity in the essential oil content of the different lines of *Agastache* spp. was observed, with the highest oil content being obtained from *A. rugosa* Ames 4992 (2.73%) and Ames 5018 (2.12%), and *A. foeniculum* Ames 7872 (2.45%). In most accessions, the flowers had a higher oil content than the leaves (Table I).

Twenty-six compounds were identified in the essential oils of the different *Agastache* species and accessions (Tables II–IV) with some of these compounds (α-camphene, β-bourbonene, bornyl acetate, *cis*-ocimene, and 7-octen-4-ol) not previously reported. There was great variation in the methylchavicol content of *A. foeniculum* lines (6.27–94.6%). The lines of *A. foeniculum* low in methylchavicol (6.27–17.9%) were high in spathulenol (10.5–49.5%). Other major oil constituents in *A. foeniculum* lines included bornyl acetate, γ-cadinene, β-caryophyllene, α-limonene, and α-cadinol (Table II). All of the *A. rugosa* lines and putative hybrids were high in methylchavicol, ranging from 46.7% to 93.7% (Tables III and IV). The other major constituent in these lines was α-limonene (2.09–14.4%) with the exception of putative hybrid Ames 5018, in which methyleugenol was also found in high concentration (15.2%). The only *A. nepetoides* line, Ames 4716, was low (6.52%) in methylchavicol (Table III). The major constituents in the essential oil of *A. nepetoides* included δ-cadinol (39.6%), spathulenol (17.4%), and β-caryophyllene (16.5%). Damascenone and β-ionone, reported to be present in *A. foeniculum* by Nykanen et al. (1989), could only be detected in *A. nepetoides* essential oil (Table III). Germacrene B was found in all *A. rugosa* lines, *A. nepetoides*, and one of the putative hybrids (Ames 5018), while caryophyllene oxide was detected only in *A. nepetoides*.

Bornyl acetate, present in three lines (Ames 4721, 4992, and 5018) of the putative hybrids, *A. nepetoides*, and all of the lines of *A. foeniculum*, was not detected in any of the *A. rugosa* lines. Similarly, α-camphene present in Ames 4992 and Ames 5018 lines of the putative hybrids and eight lines of *A. foeniculum* was absent in *A. rugosa*. Eugenol and germacrene B, which were absent in all *A. foeniculum* lines, were present both in some putative hybrid lines and in all *A. rugosa* lines. The *cis*-ocimene present in three lines of putative hybrids and *A. foeniculum* could not be detected in any *A. rugosa* lines.

The essential oil composition of the flowers of 10 lines of *A. foeniculum* and 3 populations of putative hybrids was analyzed to determine the compounds contributing to the floral scent (Table V). Methylchavicol was again the major oil component, ranging from 21.3% to 95.6%. The flower oil of *A. foeniculum* had a higher methylchavicol content than the leaf oil, while the leaf oil of putative hybrids had a higher methylchavicol content than the flower oil.

Our results demonstrate a great diversity in essential oil composition in *A. foeniculum* but low in *A. rugosa* and putative hybrids. The chemical compositions of the essential oils obtained from *A. rugosa* and hybrids were almost similar. This supports the findings of Vogelmann (1983), who found that different populations of *A. rugosa* were very similar genetically. Methylchavicol was present in all lines of *Agastache* spp. and was the major oil constituent in most accessions.

ACKNOWLEDGMENT

Journal Paper No. 12 901, Purdue University Agricultural Experiment Station, West Lafayette, IN 47907. We thank Lester A. Wilson and Jules Janick for reviewing the manuscript.

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Received for review March 4, 1991. Accepted August 7, 1991. Mention of commercial brand names does not constitute an endorsement of any product by the U.S. Department of Agriculture or cooperating agencies.

Registry No. α-Pinene, 80-56-8; α-camphene, 79-92-5; 7-octen-4-ol, 53907-72-5; β-myrcene, 123-35-3; α-limonene, 138-86-3; *cis*-ocimene, 27400-71-1; linalool, 78-70-6; dihydroterpineol, 58985-02-7; camphene hydrate, 465-31-6; isomenthone, 491-07-6; methylchavicol, 140-67-0; pulegone, 89-82-7; bornyl acetate, 76-49-3; β-bourbonene, 5208-59-3; methyleugenol, 93-15-2; β-caryophyllene, 87-44-5; δ-cadinene, 39029-41-9; δ-cadinene, 483-76-1; spathulenol, 6750-60-3; α-cadinol, 481-34-5; eugenol, 97-53-0; damascenone, 23726-93-4; β-ionone, 79-77-6; germacrene B, 15423-57-1; caryophyllene oxide, 1139-30-6; δ-cadinol, 19435-97-3.