IOWA STATE UNIVERSITY
Digital Repository

NCRPIS Publications and Papers | North Central Regional Plant Introduction Station

2000

Inheritance Studies of Aromatic Compounds in Agastache foeniculum (Pursh) Kuntze

Roger G. Fuentes-Granados
Iowa State University

Mark P. Widrlechner
United States Department of Agriculture, isumw@iastate.edu

Lester A. Wilson
Iowa State University, lawilson@iastate.edu

Follow this and additional works at: http://lib.dr.iastate.edu/ncrpis_pubs


Part of the Agricultural Science Commons, Agriculture Commons, and the Plant Breeding and Genetics Commons

The complete bibliographic information for this item can be found at http://lib.dr.iastate.edu/ncrpis_pubs/55. For information on how to cite this item, please visit http://lib.dr.iastate.edu/howtocite.html.

This Article is brought to you for free and open access by the North Central Regional Plant Introduction Station at Iowa State University Digital Repository. It has been accepted for inclusion in NCRPIS Publications and Papers by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

Inheritance Studies of Aromatic Compounds in *Agastache foeniculum* (Pursh) Kuntze[1]

Roger G. Fuentes-Granados
Iowa State University, Departments of Food Science and Human Nutrition and Agronomy
Ames, IA 50011 USA
Current address: Oklahoma State University, Department of Entomology & Plant Pathology
127 Noble Research Center, Stillwater, OK 74078 USA

Mark P. Widrlechner*
U.S. Department of Agriculture, Agricultural Research Service, North Central Regional Plant Introduction Station, Iowa State University, Department of Agronomy, Ames, IA 50011-1170 USA

Lester A. Wilson
Iowa State University, Department of Food Science and Human Nutrition, Ames, IA 50011-1061 USA

Abstract

Genetic control of volatile oil production in *Agastache foeniculum* and, more specifically, of the production of myrcene, limonene, and methyl chavicol, three major components of its essential oils, was elucidated. Controlled crosses were made between individuals with different chemotypes, and F_2 populations were analyzed for their composition of volatiles by using headspace gas chromatography. Total aromatic volatile emittance was found to be under polygenic control with additive gene effects for four of eight families studied, and each of the three major components was controlled by one to a few genes with recessive to additive effects. Evidence is also presented suggesting that population PI 561057 transmits a genetic factor or factors that suppress the overall production of the major volatile oil components.

Key Word Index

Agastache foeniculum, Lamiaceae, anise hyssop, inheritance, genetic diversity, chemotype, essential oil composition, myrcene, limonene, methyl chavicol.

Introduction

Aromatic compounds are predominantly products of secondary plant metabolism commonly found in oils isolated from leaves, fruits, flowers, and heartwoods. Biosynthesis of aromatic compounds is generally genetically controlled and results mainly from three metabolic pathways: the acetate/mevalonate and glyceraldehyde 3-phosphate/pyruvate pathways for terpenes and the shikimate

*Address for correspondence
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. β-myrcene, 123-35-3; α-limonene, 138-86-3, methyl chavicol, 140-67-0.
[1] Journal Paper No. J-18465 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA. Projects No. 1018 and 216-1. Supported by Hatch Act and State of Iowa funds and grants from the Herb Society of America and Sigma Xi. A preliminary analysis of these data was presented in a Poster Session at the 1997 Institute of Food Technologists Annual Meetings, Orlando, Florida.

Received: July 1999
Revised: December 1999
Accepted: December 1999

1041-2905/00/0005-0581$6.00/0—©2000 Allured Publishing Corp.

pathway for phenylpropanoids (1-5).

Despite the rising trend in demand for plant-derived aromatic products, little of this demand is now met by domestic agricultural production in the United States. *Agastache foeniculum* (Pursh) Kuntze, one of seven species of *Agastache* (Clayton ex Gronov.) section *Agastache* (Lamiaceae family) native to North America (6,7), is a candidate crop for expanding the domestic production of essential oils. A comprehensive review of research reports investigating the genus *Agastache* has recently been presented by Fuentes-Granados et al. (8), covering aspects of the morphology, biochemistry, and agronomy, as well as potential economic uses of the genus.

Several studies have reported the oil content and composition of *A. foeniculum* (9-12). In those studies, methyl chavicol and limonene have been reported to be major components of its oil, and myrcene a significant component of certain populations. The present study was conducted to elucidate patterns of genetic control and relationships among myrcene, limonene, and methyl chavicol, as well as to investigate the genetic control of total aromatic volatiles, among selected populations of *A. foeniculum*. The analysis of large numbers of samples at the same stage of development in a short time necessitated the use of headspace analysis for the rapid quantification of the major oil components, as demonstrated by Wilson et al. (12).

Experimental

Plant Materials: Five populations of *A. foeniculum* (Table I) were obtained from the North Central Regional Plant Introduction Station (NCRPIS), Ames, Iowa. These populations were selected for the aromatic composition of their leaves and for isozyme variability, by using staining systems known to detect polymorphic loci (13,14). Parental plants differing in aromatic composition were selected for controlled hybridization. The offspring from these crosses were analyzed and their hybridity verified by scoring the enzyme systems for which the parents differed. Table II summarizes the aromatic composition of parents and F_1 hybrids, and Table III lists the parental origin of F_2 populations. F_2 families then were evaluated for their aromatic profiles.

Plants were grown from seeds that received a moist chilling treatment, 4°C for a week, to improve germination. Seeds were germinated in a germination chamber with temperature alternating between 20°C and 30°C with a 16-h photoperiod. Two-week-old seedlings were transplanted from the germination chamber into the greenhouse. Two-month-old plants were hardened for a week before they were transplanted into a long-term field plot at the NCRPIS in early June 1995. Leaf and flower samples were collected as the plants began to flower during the month of July 1996.

Sample Preparation: To reduce variation that may occur when sampling leaves of different developmental stages, young to just fully-expanded leaf samples, totaling 8-10 g in fresh weight, were removed from individual plants as they reached anthesis. Samples were collected between 0900 and 1100 h and placed into 100 mL glass jars with special headspace sampling caps containing Teflon-coated septa and aluminum seals (15). Fresh floral samples were taken shortly thereafter following the procedure described for leaves (above). The samples were equilibrated for 1-4 h at 20°C before chromatographic analyses were conducted.

Chromatographic Analyses: Equilibrium headspace gas chromatography was used to analyze and identify the volatiles released by the leaves and inflorescences of individual plants. The chromatographic analyses were conducted on a Varian 3700 gas chromatograph equipped with an FID detector and a Hewlett-Packard 3390A integrator (Hewlett-Packard Co., Avonlea, PA). A DB-5 fused-silica capillary column (30 m x 0.25 mm) was used throughout the study. Flow rates of oxygen, hydrogen, and nitrogen (carrier gas) were 300 mL/min, 30 mL/min, and 30 mL/min, respectively. The flow of the carrier gas was partitioned in a ratio of 1 mL/min through the column to 29 mL/min as make-up gas. Oven temperatures were programmed from 40°-220°C with an increase rate of 10°C/min. A 2-min hold was set at 40°C. The sample size was 2 mL. Following Wilson et al. (12), the samples were cryofocused before the start of the temperature program. Headspace samples of standard compounds (myrcene, limonene, and

Table I. Original collection sites for the populations of *Agastache foeniculum* used in this study

Species	Accession #	Origin
A. foeniculum	PI-561057	Barnes Co., ND
A. foeniculum	PI-561058	Cass Co., MN
A. foeniculum	PI-561059	Hennepin Co., MN
A. foeniculum	PI-561061	Las Animas Co., CO
A. foeniculum	PI-561063	Manitoba

Table II. Aromatic profiles for leaves of parents and individual F_1 offspring (in %)

Individual	Myrcene	Limonene	Methyl chavicol
PI-561059	9	57	0
PI-561061	3	19	75
F_1 hybrids			
2	0	28	39
3	0	28	45
4	0	19	60
8	0	35	44
PI-561057	0	0	0
PI-561063	22	16	48
F_1 hybrids			
24	14	36	9
25	16	47	7
26	13	45	6
PI-561058	59	15	25
PI-561059	9	57	0
F_1 hybrids			
60	32	35	0
62	29	34	0
72	28	37	0
84	58	21	0

Table III. Parental origin of the F_2 families evaluated

F_2 Family	Original Cross	F_1 Parents
1	PI-561061 x PI-561059	F_1 (3) x F_1 (2)
2	PI-561061 x PI-561059	F_1 (4) x F_1 (3)
3	PI-561061 x PI-561059	F_1 (8) x F_1 (4)
4	PI-561063 x PI-561057	F_1 (25) selfed
5	PI-561063 x PI-561057	F_1 (26) x F_1 (24)
6	PI-561059 x PI-561058	F_1 (60) x F_1 (84)
7	PI-561059 x PI-561058	F_1 (62) x F_1 (60)
8	PI-561059 x PI-561058	F_1 (72) x F_1 (62)

methyl chavicol) were used to verify the identity of the compounds.

GC/MS analyses were conducted as an independent verification of the identities of myrcene, limonene, and methyl chavicol. Specifically for these analyses, five to seven unblemished, mature leaves were cut from the stems for sampling and transferred to a glass-volatile stripping apparatus (16) for dynamic headspace isolation of volatile compounds. The apparatus was flushed with helium at 75 mL/min

for 30 min. The volatiles stripped from the leaves were trapped in a 3 mm o.d. x 72 mm glass tube filled with Tenax TA (Alltech Assoc., Deerfield, IL) as an adsorbent.

Volatile compounds were thermally desorbed from the Tenax-packed tubes, located inside the injector port of a Hewlett-Packard Model 5890A gas chromatograph (Hewlett-Packard Co., Avondale, PA), at 230°C, transferred in helium at 1.5 mL/min onto a SPB-1 fused-silica capillary column (30 m x 0.25 mm, 0.25 μm film thickness) (Supelco, Bellefonte, PA), and condensed in the first loop of the column, which was cooled in a dry ice bath (16). After exactly 5 min of desorption and transfer, the dry ice bath was removed and the temperature was held for 3 min at 40°C and raised from 40°-250°C at 10°C/min. Mass spectra were recorded in the electron impact mode in a Hewlett-Packard 5970 mass selective detector (Hewlett-Packard Co., Avonlea, PA). Peaks were identified by comparing their retention times and mass spectra with those of known compounds.

Statistical Analysis: The total integration area and those of the major peaks were standardized to reflect the fresh weight of each sample and are expressed herein as standardized integration units. Related F_2 families resulting from the same original hybridization event were combined for statistical analysis when shown to be homogeneous for a particular character. The Kruskal-Wallis and the Rank-Sum-Z tests were used to measure homogeneity among related families (17). Homogeneity was rejected when test statistics exceeded the $p = 0.1$ level. Then, standardized data were analyzed for segregation tests of Fixed-Ratio Hypotheses, assuming single gene models, by using the Chi-square test. The criteria for assigning values to classes were developed by considering parental values and after examining plots of the progenies' values for each major component under investigation. These criteria are described in greater detail as part of the Results and Discussion. Analyses of Variance and regression analyses of the standardized data were conducted using the Proc Anova and Proc Reg procedures of SAS (SAS Institute, Inc., Cary, NC).

Results and Discussion

Many factors, both genetic and environmental, can contribute to the overall production of essential oils. Genetic factors may influence both production levels within specific metabolic pathways and the size and density of secretory structures.

Figures 1-4 present bar graphs of standardized quantities of total volatiles, myrcene, limonene, and methyl chavicol, respectively, including all F_2 individuals evaluated for aromatic composition, grouped by statistically homogeneous families (as described in the Experimental section).

Total Volatiles: Related F_2 families, 1 and 2, 4 and 5, and 6, 7, and 8, were found to be statistically homogeneous for total volatile content. Only family 3 differed significantly in the pattern of its total volatiles from related families 1 and 2 and thus was analyzed separately (Figure 1).

We hypothesized that the genetic control of the quantity of total volatiles emitted was a polygenic trait influenced by many genes with additive effects, including both genes involved in the relevant biochemical pathways and those that control the density and size of secretory structures. If correct, the distribution of total volatile production should be predicted by a normal distribution with the mean and standard deviation estimated by our data sets. Data were classified in a fashion that combined minor classes where expected values were <1.0, such that all expectations were >1.0, and were then subjected to chi-square analysis. Our hypothesis of polygenic control was supported only for family 3 and for families 6, 7, and 8. The hypothesis was rejected for the other families at the 0.01 significance level.

For the other families, we discovered that one or two of the major components were the primary determinants of total volatile content. For families 1 and 2, the combined value of limonene and methyl chavicol was highly correlated with the value for total volatiles ($r^2 = 0.94$, $p < 0.001$); and for families 4 and 5, limonene content was highly correlated with the value for total volatiles ($r^2 = 0.72$, $p < 0.001$). In these cases, one or a few genes with major effects can overwhelm genetic variation for other contributing characteristics.

Myrcene: Myrcene is a commonly found acyclic monoterpene with a sweet, balsamic odor (18).

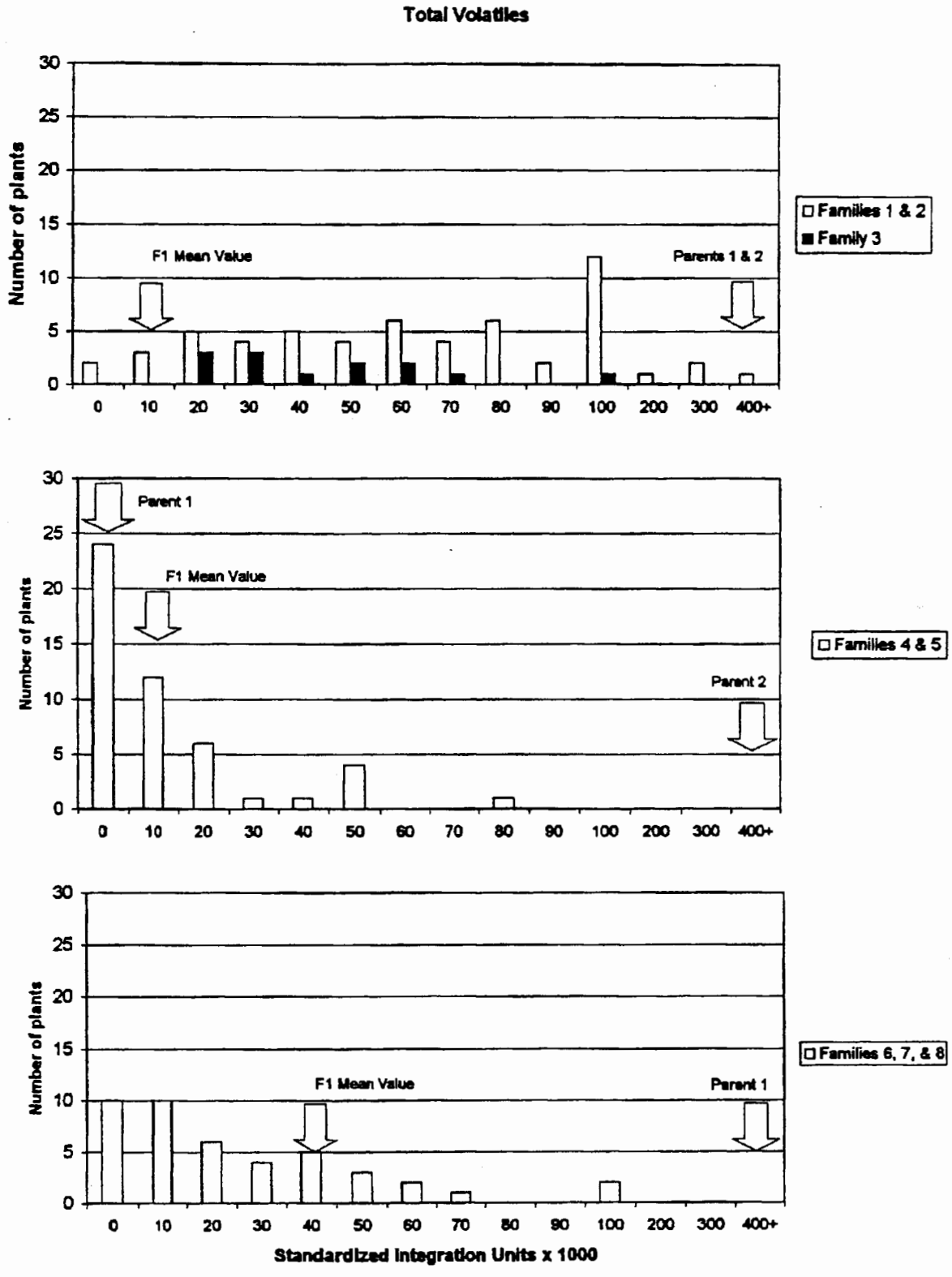

Figure 1. Distribution of levels of total volatiles in nine F_2 families of *Agastache foeniculum* (measured as standardized integration units)

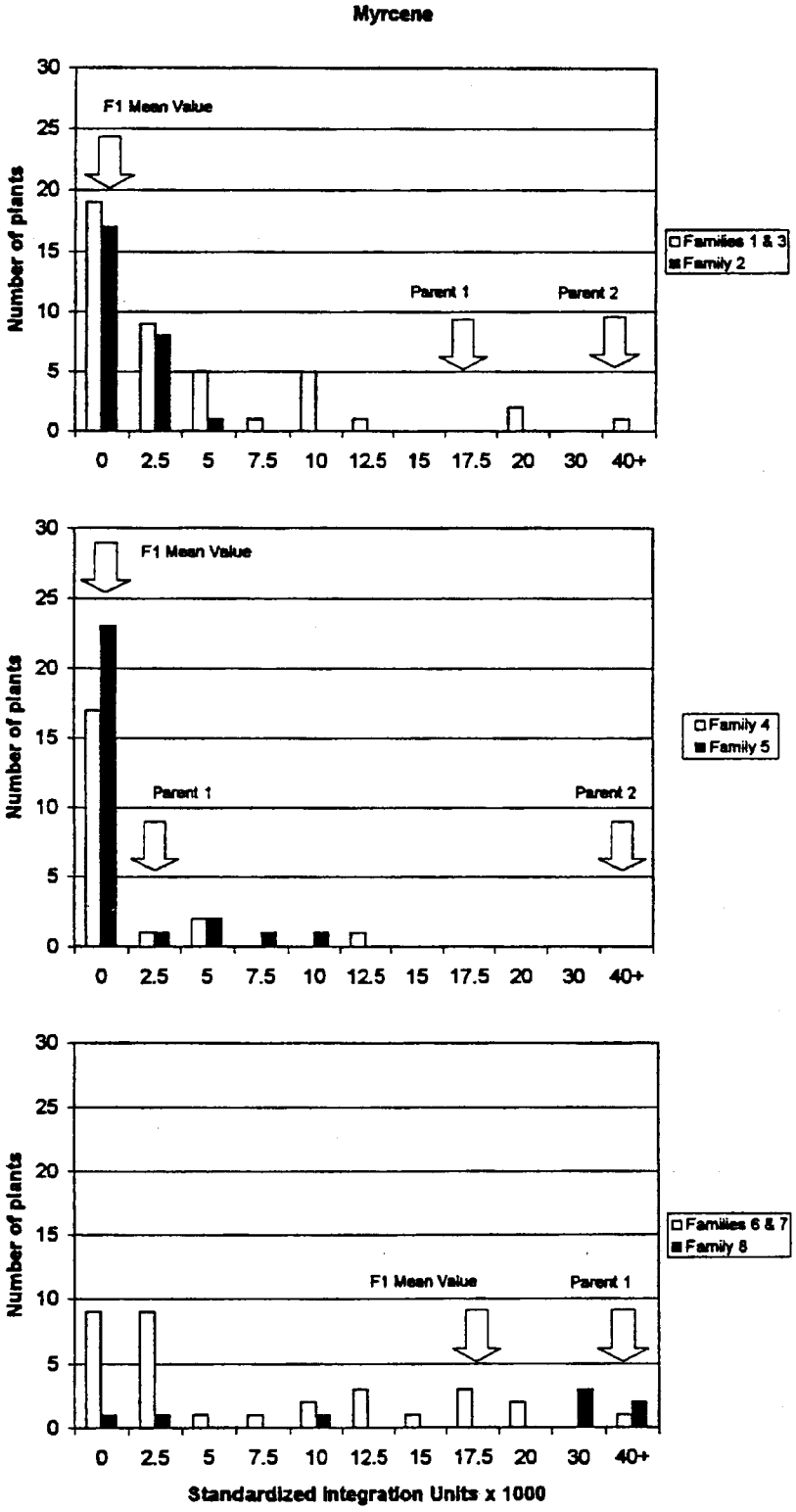

Figure 2. Distribution of myrcene levels in nine F_2 families of *Agastache foeniculum* (measured as standardized integration units)

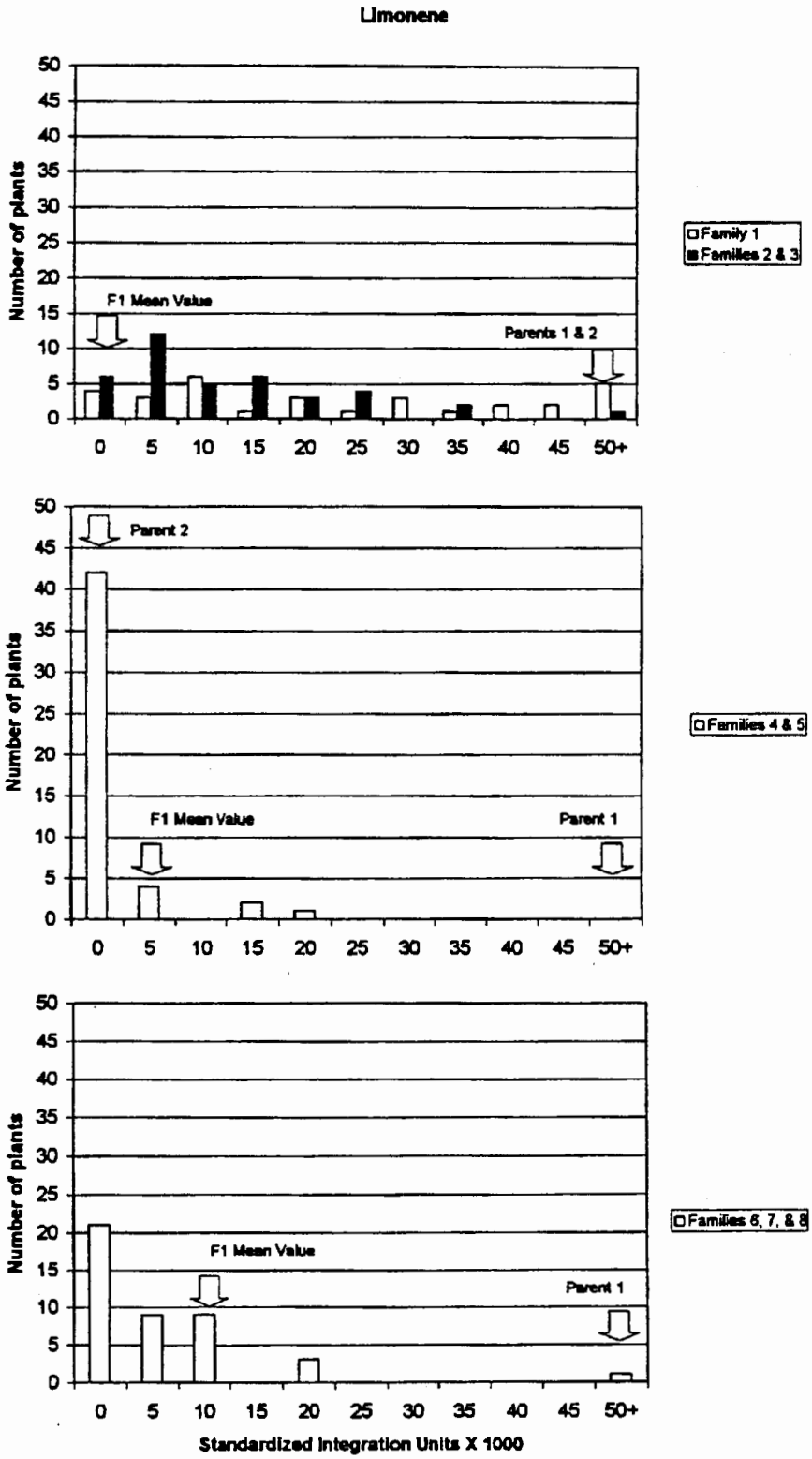

Figure 3. Distribution of limonene levels in nine F_2 families of *Agastache foeniculum* (measured as standardized integration units)

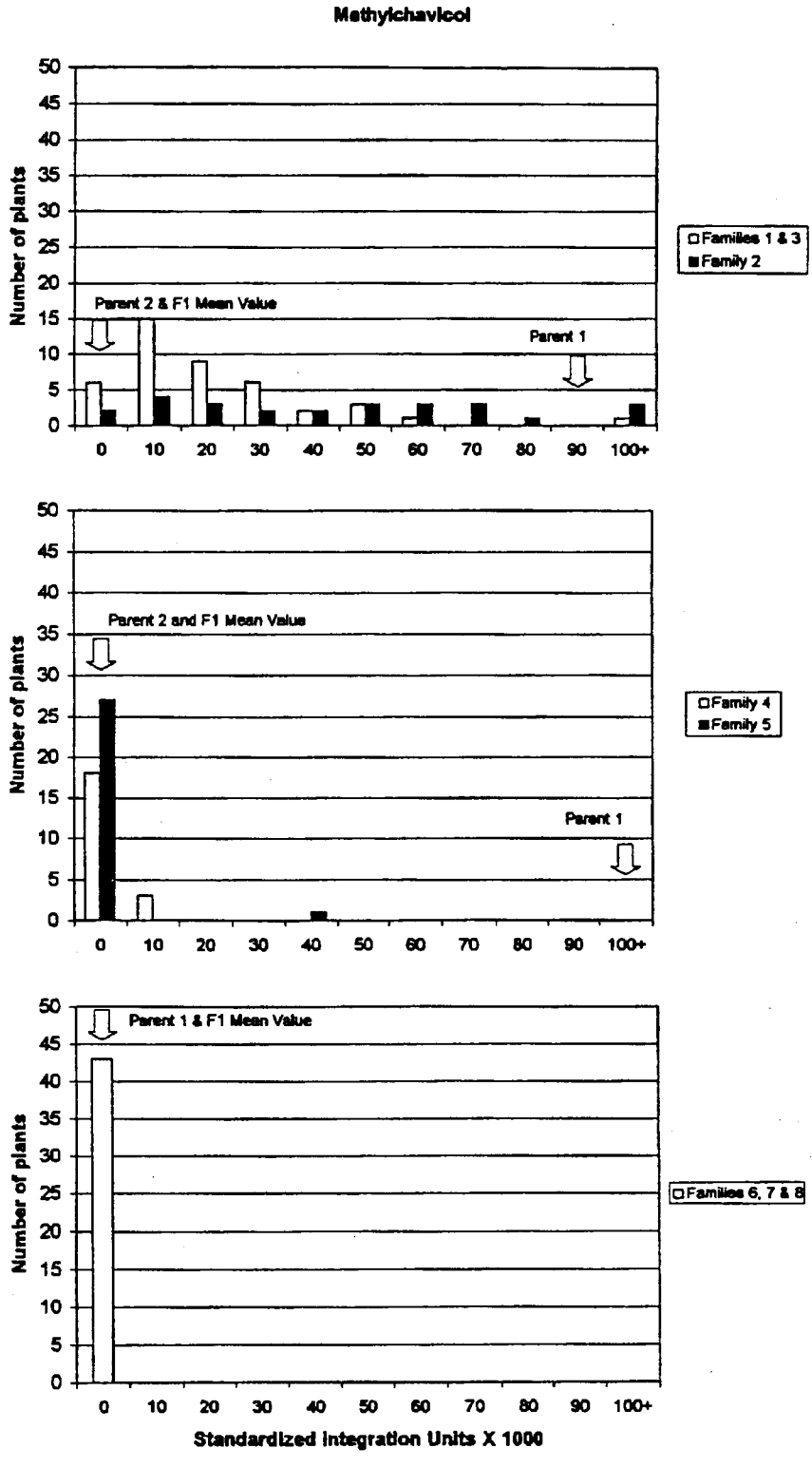

Figure 4. Distribution of methyl chavicol levels in nine F_2 families of *Agastache foeniculum* (measured as standardized integration units)

In *Pinus*, myrcene has been reported to be controlled by a single gene with dominance for its high content in cortical oleoresin (19).

We evaluated three segregating F_2 populations (families 1-3) from the initial cross PI-561059 x PI-561061, which represented the hybridization of chemotypes with moderate and low myrcene contents. The F_1 progeny exhibited little or no myrcene production, suggesting that myrcene production should have been under recessive genetic control in these populations. Families 1 and 3 were statistically homogeneous and thus combined for analysis.

We also evaluated two segregating F_2 populations (families 4 and 5) from the initial cross PI-561057 x PI-561063, which represented the hybridization of a null foliar chemotype by a high myrcene chemotype. The F_1 progeny exhibited low levels of foliar myrcene production, suggesting that myrcene production was under additive genetic control in these populations. Families 4 and 5 were statistically heterogeneous and analyzed separately.

Furthermore, we additionally evaluated three segregating F_2 populations (families 6-8) from the initial cross PI-561058 x PI-561059, which represented the hybridization of chemotypes with high and moderate myrcene. The F_1 progeny exhibited low, moderate, and high levels of foliar myrcene production, suggesting that myrcene production was under additive genetic control. Families 6 and 7 were statistically homogeneous and thus combined for analysis.

Data for foliar myrcene production in these eight families suggested that there may be gaps in the distribution of these data that may provide at least putative classes (Figure 2). All populations exhibited gaps that likely mark the difference between low and moderate myrcene production. At the lowest, in families 4 and 5, a gap was observed between 3,000 and 5,000 standardized integration units (SIU), and, at the other extreme, in families 6 and 7, between 7,000 and 9,500 SIU. Families 1, 3, 6, 7, and 8 also exhibited gaps in a range between about 20,000 and 30,000 SIU that may mark the difference between moderate and high classes. Families 2, 4, and 5 included no members of the putative high class.

For families 1-3, we hypothesized that these families should segregate in a 3:1 (low: moderate) ratio. Families 1 and 3, analyzed jointly, presented a 33:8:2 (low: moderate: high) ratio, which, except for the presence of two transgressive segregants, fits the hypothetical expectation of 32:11. Family 2 presented a 26:0 (low:moderate) ratio, suggesting that the moderate parent was actually heterogeneous for this trait and did not pass the gene(s) for moderate myrcene levels to the specific parents of this family.

For families 4 and 5, we hypothesized that these families should segregate in a 1:2:1 (low: moderate: high) ratio. But this was completely different from the observed values of 18:3:0 for family 4 and 24:4:0 for family 5. Five of 18 of the low values for family 4 and 15 of 24 of the low values for family 5 were recorded as possessing no detectable myrcene. When categorized in a classification of no: low: moderate, there was a somewhat closer conformity to a 1:2:1 ratio; family 4 presented a 5:13:3 (no: low: moderate) ratio (chi-square with 2 d.f. = 1.57, n.s.), and family 5 presented a 15:9:4 (no: low: moderate) ratio (chi-square with 2 d.f. = 12.21, $p < 0.01$). However, even with this alteration, which would reflect a diminished production of myrcene relative to our original expectation, family 5 had a much higher than expected number of myrcene-null plants. This phenomenon of greatly diminished aromatic volatile emittance in families 4 and 5 also was observed for limonene and for methyl chavicol (discussed below), and it may reflect a partially dominant gene or genes from PI 561057 that inhibited oil production.

For families 6-8, we hypothesized that these families should segregate in a 1:2:1 (low: moderate: high) ratio. Families 6 and 7 presented a 19:12:1 (low: moderate: high) ratio, significantly different from our expectation (chi-square with 2 d.f. = 22.25, $p < 0.01$). In agreement with our hypothesis, family 8 presented a 2:1:5 (low:moderate:high) ratio (chi-square with 2 d.f. = 4.75, n.s.), but it was difficult to draw any conclusion about this family given its small size.

It is likely that an appropriate method to produce high myrcene chemotypes of *A. foeniculum* would be to select first within population PI 561063. None of the 158 F_2 hybrids presented myrcene levels as high as those found in that parental population, although there were a few individual plants in families 7 and 8 that were rated as high in myrcene and low to moderate in limonene and methyl chavicol.

Limonene: Limonene is the first cyclic monoterpene synthesized from geranyl pyrophosphate (2). In *Pinus*, limonene production has been reported to be controlled by a single locus with dominant gene action for its production (19), while in *Mentha* and *Perilla*, limonene control is more complex (20,21). The accumulation of limonene in *Mentha* is controlled by a dominant allele closely linked to recessive allele which eliminates the oxygenation of limonene (20). In *Perilla*, limonene is controlled by the interaction of two dominant genes through the gene products, geranyl pyrophosphate synthase and limonene synthase (21).

We evaluated three segregating F_2 populations (families 1-3) from the initial cross PI-561059 x PI-561061, which represented the hybridization of chemotypes with high and moderate limonene contents. The F_1 progeny exhibited low levels of limonene, suggesting that limonene production was under recessive genetic control in these populations. Families 2 and 3 were statistically homogeneous and thus combined for analysis.

We also evaluated two segregating F_2 populations (families 4 and 5) from the initial cross PI-561057 x PI-561063, which represented the hybridization of a null foliar chemotype by a moderate limonene chemotype. The F_1 progeny exhibited low to moderate levels of foliar limonene production, suggesting that limonene production was under additive genetic control in these populations. Families 4 and 5 were statistically homogeneous and thus combined for analysis.

Additionally, we evaluated three segregating F_2 populations (families 6-8) from the initial cross PI-561058 x PI-561059, which represented the hybridization of chemotypes with low and high limonene contents. The F_1 progeny exhibited low to moderate levels of foliar limonene production, suggesting that limonene production was under recessive to additive genetic control. Families 6-8 were statistically homogeneous and thus combined for analysis.

Data for foliar limonene production suggested that there may be gaps in their distribution that may provide at least a putative classification (Figure 3). Families 1-3 presented a continuous distribution of low values with a gap noted in their distribution between 37,000 and 42,500 SIU. Families 4 and 5 generally presented very low levels of production with two gaps noted, one between 3,300 and 4,900 SIU and another between 8,000 and 15,000 SIU. The putative division of plants from families 6-8 into classes was somewhat problematic: there was a major gap in the distribution between 15,000 and 20,000 SIU and one plant in family 7 that had much higher production (75,986 SIU) than all the other plants in these three families.

For families 1-3, we hypothesized that these families should segregate in a 3:1 (low to moderate: high) ratio. Families 2 and 3, analyzed jointly, presented a 38:1 (low to moderate: high) ratio, which deviates widely (chi-square with 1 d.f. = 10.83, $p < 0.01$) from the hypothetical expectation of 28.75:9.75, suggesting that the high parent was actually heterogeneous for this trait and did not pass the gene(s) for high limonene levels to the specific parents of these families. Family 1 presented a 22:9 (low to moderate: high) ratio, in agreement with our hypothesis (chi-square with 1 d.f. = 0.32, n.s.).

For families 4 and 5, we hypothesized that these families should segregate in a 1:2:1 (low: moderate: high) ratio. However, we observed values of 46:3:0. Twelve of 46 of the low values for families 4 and 5 were recorded as zero, with no detectable limonene. When categorized as no: low: moderate, there was somewhat closer conformity to a 1:2:1 ratio; families 4 and 5 presented a 12:34:3 (no: low: moderate) ratio (chi-square with 2 d.f. = 11.57, $p < 0.01$). However, even this alteration, which would reflect a diminished production of limonene relative to our original expectation, did not explain the deficiency in plants with moderate limonene production.

For families 6-8, we hypothesized that these families should segregate in a 1:2:1 (low: moderate: high) ratio, if additive gene action were predominant. Families 6-8 presented a 35:3:1 (low:moderate:high) ratio, widely different than our expectation (chi-square with 2 d.f. = 87.21, $p < 0.01$). If recessive gene action were predominant, these families should segregate in a 3:1 (low: moderate) ratio. This hypothesis was rejected, in this case at the 0.05 level (chi-square with 1 d.f. = 4.52), but it may be that the division between the low and moderate classes occurred not at the gap between 15,000 and 20,000 SIU, but at

another, lower level.

These results suggested that high limonene chemotypes of *A. foeniculum* can be selected within population PI 561059. Charles et al. (9) reported that this population (described therein as Ames 7611) exhibited the highest foliar limonene concentration of the ten populations evaluated. None of the 158 F_2 hybrids presented limonene levels as high as those found in that parental population, although there were a few individual plants in family 1 that were rated as high in limonene.

Methyl Chavicol: Methyl chavicol, a phenylpropanoid compound synthesized via the shikimate pathway, has an anise-like aroma with a sweet flavor (22) and is found in a broad array of herbs and spices (23). It is generally the predominant component of *A. foeniculum* oil (9-12). In *Ocimum basilicum* and *O. americanum*, it was reported to be inherited as a single Mendelian trait with dominance for its production (24,25).

We evaluated three segregating F_2 populations (families 1-3) from the initial cross PI-561059 x PI-561061, which represented the hybridization of a high limonene: moderate myrcene foliar chemotype (with no methyl chavicol) by a high methyl chavicol chemotype. The F_1 progeny exhibited low methyl chavicol levels, suggesting that this compound was under recessive to additive genetic control in these populations. Families 1 and 3 were statistically homogeneous and thus combined for analysis.

We also evaluated two segregating F_2 populations (families 4 and 5) from the initial cross PI-561057 x PI-561063, which represented the hybridization of a null foliar chemotype by a high methyl chavicol chemotype. The F_1 progeny exhibited low levels of foliar methyl chavicol production. These results also suggested that methyl chavicol production was under recessive to additive genetic control. Families 4 and 5 were statistically heterogeneous and analyzed separately.

Additionally, we evaluated three segregating F_2 populations (families 6-8) from the initial cross PI-561058 x PI-561059, which represented the hybridization of a low methyl chavicol chemotype by a high limonene: moderate myrcene chemotype (with no methyl chavicol). The F_1 progeny produced no detectable methyl chavicol, suggesting that methyl chavicol production in this case was under strict recessive genetic control. Families 6-8 were statistically homogeneous and thus combined for analysis.

A close examination of methyl chavicol data (Figure 4) suggested that there may be gaps in the distribution of these data for families 1-3 that could provide at a putative classification. All other families were strongly weighted toward very low levels of foliar methyl chavicol. An examination of the distribution of data for families 1 and 3 indicated a statistically normal distribution up to 61,000 SIU. For family 2, there was a gap between 64,000 and 73,000 SIU. These gaps can be used to mark the difference between putative moderate and high classes. No gaps were observed between putative low and moderate classes.

Our expectation for families 6-8 would be that all plants would be in the zero or low classes. The highest value for foliar methyl chavicol observed among the 43 progeny in these three families was 5,529 SIU, and the highest F_1 value observed was 7,446 SIU. We would expect that the division between the low and moderate classes would occur between 7,500 and 35,000 SIU (the upper limit at approximately half the level dividing the moderate and high classes).

Under a recessive model, families 1-5 should segregate in a 3:1 (low: moderate) ratio, but under an additive model, these families would segregate in a 1:2:1 (low: moderate: high) ratio. Families 1 and 3, analyzed jointly, presented a 43:1 (low to moderate: high) ratio, and family 2 presented a 19:7 (low to moderate: high) ratio. Families 4 and 5 both were comprised exclusively of plants in the low to moderate range. Our data for families 1 and 3 seemed to give some support to the recessive hypothesis. If one arbitrarily divides the data for these families into low and moderate classes at 34,000 SIU, the data fall into three classes 32:10:1 (low: moderate: high), which, except for the presence of a single high outlier, would be almost exactly the ratio predicted by the recessive hypothesis. However, our data for family 2 gave greater support to the additive hypothesis. If one divides the data for this family into low and moderate classes at anywhere between 30,000 and 35,000 SIU, the data then fall into three classes, 9:10:7

Table IV. Regression analyses among the content of myrcene, limonene and methyl chavicol in leaves and in flowers of *Agastache foeniculum*

Leaves	F-value	P-value	R-square
Myrcene - Limonene	6.21	0.014	0.045
Myrcene - Methyl chavicol	0.92	0.34	0.007
Limonene - Methyl chavicol	1.07	0.30	0.008
Flowers	**F-value**	**P-value**	**R-square**
Myrcene - Limonene	19.81	0.0001	0.131
Myrcene - Methyl chavicol	6.2	0.014	0.045
Limonene - Methyl chavicol	0.871	0.35	0.007

(low: moderate: high), which is not statistically different from expectations under the additive model (chi-square with 2 d.f. = 1.69, n.s.).

As noted earlier in our discussions of myrcene and limonene, the production of methyl chavicol in families 4 and 5 was much less than that predicted by even the recessive model. Family 4 had no plants with foliar methyl chavicol levels above 14,000 SIU, and family 5 had only one plant with a value above 7,600 SIU, although for both families one would expect that one quarter of their plants should be in the moderate class (30,000 to 70,000 SIU), providing additional evidence for genetic suppression of aromatic volatile production inherited from PI 561057, the null parent.

For families 6-8, we hypothesized that these families should segregate in a 3:1 (zero: low) ratio. Families 6-8 presented a 5:38 (zero: low) ratio, significantly different from our expectation (chi-square with 1 d.f. = 89.05, $p < 0.01$). This seemingly wide discrepancy could easily be explained if F_1 plants were producing such low levels of methyl chavicol that they were below the level of detection during our initial evaluations, and were thus incorrectly classified. Nine of the F_2 progeny were noted with foliar methyl chavicol levels below 1,000 SIU, very close to our lowest confident detection levels.

On the basis of our results, an appropriate method to produce high methyl chavicol chemotypes of *A. foeniculum* would be to select within population PI 561063. None of the 158 F_2 hybrids presented methyl chavicol levels as high as those found in that parental population, although there were a few individual plants in families 1 and 2, unrelated to PI 561063, that were rated as high in methyl chavicol and low to moderate in myrcene and moderate to high in limonene. The high methyl chavicol parent of families 1 and 2 is PI 561061, which, based on the report by Charles et al. (9) (population described therein as Ames 7872) of 2.45% total essential oil and 92.6% methyl chavicol, may also be an excellent base population for further selection.

Relationships among Oil Components: We conducted linear regression analyses between the three paired combinations of myrcene, limonene, and methyl chavicol to determine if there were correlations between any of these compounds (Table IV). Regression analyses of all possible pairs of compounds from headspace samples of both leaves and flowers revealed significant positive correlations between myrcene and limonene, $p = 0.0139$ and 0.0001, for their relationships in leaves and flowers, respectively. In contrast, one negative correlation was identified between myrcene and methyl chavicol in flowers. Significant correlations could indicate linkages between the genes responsible for the synthesis of those compounds, but the amount of variation explained by even the strongest of those correlations, as described by their r^2 values (Table IV), was too small to consider any of those correlations of biological significance.

We conducted analyses of variance to compare the mean amounts of myrcene, limonene, methyl chavicol, and the total volatiles given off by leaves with that produced by floral tissue. For all of the variables, myrcene, limonene, methyl chavicol, and total volatiles, the amount of compounds produced in flowers was higher than in leaves (Table V). Differences in the content and composition of the

Table V. Comparison of the mean contents (reported as SIU) of myrcene, limonene, methyl chavicol and total volatiles in leaves versus flowers in *Agastache foeniculum*

Compound	Leaves	Flowers	F-value	P-value
Myrcene	4914	82907	28.78	0.0001
Limonene	12025	97906	26.40	0.0001
Methyl chavicol	18403	142582	82.24	0.0001
Total volatiles	41215	372002	118.37	0.0001

volatiles from leaves and flowers have previously been reported in *Agastache* species (9,12), with Charles et al. (9) reporting that, in eight of ten *A. foeniculum* populations tested, the floral oil content exceeded that of the leaves.

Overview of Genetic Effects: Our hypothesis of polygenic control of total volatile production in the leaves of *A. foeniculum* was supported in four of eight families analyzed. In the other four families, total volatiles were so closely correlated with the production of one or two individual compounds that a small number of genes controlling the biosynthesis of those compounds could overwhelm other genetic factors contributing to overall production.

Based on preliminary data collected from a small number of F_1 progeny, we hypothesized that the production of myrcene, limonene, and methyl chavicol should generally be under either recessive or additive (co-dominant) genetic control. Although we noted statistically significant correlations between levels of myrcene and limonene in certain families, the proportion of variation explained by these correlations was so low as to indicate that genetic control of these factors is effectively independent.

In 7 of the 24 cases, F_2 segregation data clearly supported our initial hypotheses. But there were three important instances where our hypothetical expectations were unmet. The most significant was in the six cases involving families 4 and 5. There was evidently a partially dominant factor or factors inherited from population PI 561057 that inhibited the production of most, if not all, volatile oil constituents. This population was reported (as Ames 4550) to be a low oil (0.36% vol/dry wt), high spathulenol (45.6%) chemotype by Charles et al. (9). A second exceptional situation occurred in family 2 for myrcene and families 1 and 3 for limonene, where it was likely that one of the parental lines was heterozygous for genes controlling production and only passed on to those F_2 families recessive factors effecting low levels of production. And finally, there were cases where it was likely that actual genetic control involves more than one gene, or that gene action lies between classical recessive and co-dominant levels.

In contrast to findings for other mint-family plants, such as *Mentha*, *Ocimum*, and *Perilla* (5,20,21,24-26), we found no evidence for dominant genes controlling the biosynthetic pathways or levels of production for oils in *A. foeniculum*, with the possible exception of a partially dominant inhibitory effect derived from PI 561057. This suggests that *A. foeniculum* chemotypes with high production of particular oils may be fixed for specific genes with recessive to additive action and that it may generally be more productive to make selections within populations than to expect highly productive types through interpopulational hybridization. This would be more consistent with reports of recessive to additive genetic control of menthofuran production in *Mentha* (27) and of myristicin production in *Perilla* (28), and of two complementary recessive factors controlling geranial production in *Perilla* (29).

For further clarification of the genetic control of *A. foeniculum* oils, experiments should be conducted to investigate the stability of oil production throughout the growing season and under various environmental conditions. In addition, we suspect that the statistical analysis of segregating populations would be more straightforward if partially inbred lines, fixed for particular chemotypes, would be used as parental lines.

Acknowledgment

We wish to thank Eran Pichersky and James Simon for their useful critiques of this manuscript.

References

1. R. Croteau, *Metabolism of monoterpenes in mint (Mentha) species*. Planta Med., **57**, 10-14 (1991).
2. T. Endo and T. Suga, *Demonstration of geranyl diphosphate synthase in several higher plants*. Phytochemistry, **31**, 2273-2275 (1992).
3. H. K. Lichtenthaler, M. Rohmer and J. Schwender, *Two independent biochemical pathways for isopentenyl diphosphate and isoprenoid biosynthesis in higher plants*. Physiol. Plantarum, **101**, 643-652 (1997).
4. M. J. Murray, D. E. Lincoln and P. M. Marble, *Oil composition of Mentha aquatica X M. spicata F1 hybrids in relation to the origin of M. piperita*. Can. J. Genet. Cytol., **14**, 13-29 (1972).
5. A. Nishizawa, G. Honda and M. Tabata, *Determination of the final steps of biosynthesis of essential oil components in Perilla frutescens*. Planta Med., **55**, 251-253 (1989).
6. H. Lint and C. Epling, *A revision of Agastache*. Amer. Midl. Nat., **33**, 207-230 (1945).
7. J. E. Vogelmann, *Flavonoids of Agastache section Agastache*. Biochem. Syst. Ecol., **12**, 363-366 (1984).
8. R. G. Fuentes-Granados, M. P. Widrlechner and L. A. Wilson, *An overview of Agastache research*. J. Herbs Spices Med. Plants, **6**, 69-97 (1998).
9. D. J. Charles, J. E. Simon and M. P. Widrlechner, *Characterization of essential oils of Agastache species*. J. Agric. Food Chem., **39**, 1946-1949 (1991).
10. G. Mazza and F.A. Kiehn, *Essential oils of Agastache foeniculum, a potential source of methylchavicol*. J. Essent. Oil Res., **4**, 295-299 (1992).
11. K. P. Svoboda, J. Gough, J. Hampson and B. Galambosi, *Analysis of the essential oils of some Agastache species grown in Scotland from various seed sources*. Flav. Fragr. J., **10**, 139-145 (1995).
12. L. A. Wilson, N. P. Senechal and M. P. Widrlechner, *Headspace analysis of the volatile oils of Agastache*. J. Agric. Food Chem., **40**, 1362-1366 (1992).
13. R. G. Fuentes-Granados and M. P. Widrlechner, *Diversity among and within populations of Agastache foeniculum*. In: *Proceedings of the 14th North American Prairie Conference*. Edit., D. Hartnett, pp 1-8, Kansas State University, Manhattan, KS (1995).
14. R. G. Fuentes-Granados, M. P. Widrlechner and L. A. Wilson, *Allozyme inheritance in anise hyssop [Agastache foeniculum (Pursh) Kuntze] (Lamiaceae)*. J. Amer. Soc. Hort. Sci., **123**, 868-874 (1998).
15. G. Ong, *The Influence of Packaging, Temperature, and Light on the Color and Flavor Retention of Paprika, Rosemary, Thyme, and Tarragon*. M.S. Thesis, Iowa State University, Ames, IA (1988).
16. I. Lee, S. H. Fatemi, E. G. Hammond and P. J. White, *Quantitation of flavor volatiles in oxidized soybean oil by dynamic headspace analysis*. J. Amer. Oil Chem. Soc., **72**, 539-546 (1995).
17. J. D. Gibbons, *Nonparametric Methods for Quantitative Analysis*. Holt, Rinehart and Winston, New York (1976).
18. Committee on Food Chemicals Codex, *Food Chemicals Codex*. 4th ed. National Academy Press, Washington, DC (1996).
19. A. E. Squillace, O. O. Wells and D. L. Rockwood, *Inheritance of monoterpene composition in cortical oleoresin of loblolly pine*. Silvae Genetica, **29**, 141-151 (1980).
20. R. Croteau and J. Gershenzon, *Genetic control of monoterpene biosynthesis in mints (Mentha: Lamiaceae)*. Recent Adv. Phytochemistry, **28**, 193-229 (1994).
21. A. Nishizawa, G. Honda and M. Tabata, *Genetic control of the enzymatic formation of cyclic monoterpenoids in Perilla frutescens*. Phytochemistry, **31**, 139-142 (1992).
22. R. A. Hussain, L. J. Poveda, J. M. Pezzuto, D. D. Soejarto and A. D. Kinghorn, *Sweetening agents of plant origin: phenylpropanoid constituents of seven sweet-tasting plants*. Econ. Bot., **44**, 174-182 (1990).
23. D.L.J. Opdyke, *Monographs on fragrance raw materials*. Food Cosmet. Toxicol., **14**, 601-633 (1976).
24. S. C. Gupta and S. N. Sobti, *Inheritance pattern of methyl chavicol and citral in Ocimum americanum*. Indian Perfum., **34**, 253-259 (1990).
25. P. Pushpangadan, S.N. Sobti and R. Khan, *Inheritance of major essential oil constituents in Ocimum basilicum var. glabratum Benth. (French basil)*. Indian J. Exptl. Biol., **13**, 520-521 (1975).
26. D. E. Lincoln and M. J. Murray, *Monogenic basis for reduction of (+)-pulegone to (–)-menthone in Mentha oil biogenesis*. Phytochemistry, **17**, 1727-1730 (1978).
27. M. J. Murray and F. W. Hefendehl, *Changes in monoterpene composition of Mentha aquatica produced by gene substitution from M. arvensis*. Phytochemistry, **11**, 2469-2474 (1972).
28. Y. Koezuka, G. Honda and M. Tabata, *Genetic control of polypropanoids in Perilla frutescens*. Phytochemistry, **25**, 2085-2087 (1986).
29. G. Honda, A. Yuba, A. Nishizawa and M. Tabata, *Genetic control of geranial formation in Perilla frutescens*. Biochem. Genetics, **32**, 155-159 (1994).