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EFFECT OF DEVELOPMENT ON MONOTERPENE COMPOSITION OF *HEDEOMA DRUMMONDII*

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Key Word Index—*Hedeoma drummondii*; Labiatae; chemotaxonomy; monoterpenes; non-genetic variation.

Abstract—Samples of leaves, flowers and whole plants were taken from clonal stock of *Hedeoma drummondii* to determine the effect of developmental age on the monoterpene profile. GLC analysis revealed that there are significant differences in the quantity of major monoterpenes in leaves and flowers of different ages and in plants at different flowering stages. The results are discussed in relation to biogenetic pathways and implications for taxonomic work.

INTRODUCTION

Studies of the non-genetic variation of monoterpenes have increasingly aided in the interpreting of field-gathered data for taxonomic work as well as in indicating possible biosynthetic relationships of various compounds. To increase our understanding of quantitative variation found in several species of *Hedeoma* in the field, the effects of the developmental age of various plant organs on the monoterpene profile of *Hedeoma drummondii* var. *drummondii* Benth. were studied. The work was accomplished through standard procedures of steam distillation and direct induction, the latter procedure enabling us to analyse the changes in specific leaves and flowers. The material for this study was obtained from clonal specimens grown under stable, controlled conditions.

Other workers have variously reported both the presence and lack of quantitative shifts due to developmental changes [1–6]. The nature of the phenomenon apparently is specific to the taxa being studied and bears investigation prior to making any taxonomic interpretation from monoterpene data.

RESULTS AND DISCUSSION

Hedeoma drummondii (Labiatae) is a weedy, herbaceous, mostly annual species. Its range includes most of the intermountain west from Montana south to northern Mexico. The volatile oils have been previously analysed as an aid to understanding patterns of introgression between this species and several other closely related taxa [7]. The typical composition of the principal monoterpenes of *H. drummondii* obtained through steam distillation of the entire plant is shown in Table 1.

A group of clonal plants were analysed by whole plant steam distillation at intervals as they grew from seedlings through flowering, and some changes in terpene composition were observed. The induction technique demonstrated, however, that the difference was probably due to the age of various organs rather than shifts in composition triggered by the onset of flowering as has been reported for some other species [8–10]. When single, mature leaves were analysed from a plant through the development period, there were no detectable changes attributable to flowering. As leaves on a given stem were analysed progressing from the growing tip downward, however, there was a steady shift in the quantitative levels of several of the major compounds (Table 2). This general pattern was the same both before and after flowering. The changes occur most rapidly at two points. First, there is a large shift between the bud and the fourth leaf pair proceeding downward on the stem. The bud itself has been found to contain (+)-limonene comprising as much as 40% of the total oil, whereas it is a minor constituent in the fully developed leaf. This early build up and then decline (reflected both in per cent and actual quantity) agree with data published by von Rudloff and Zavarin in analyses of diverse plant taxa [11, 12], and

Table 1. Composition of the oil of clones 755-1 and BT1 (*H. drummondii*)

Peak	Peak combinations for statistical analysis	Identity	Per cent composition of total oil
1		α-pinene	0.35
3		camphene	0.01
4		β-pinene	0.31
6		(myrcene)	0.33
7		(+)-limonene	2.0
14			0.21
15		menthone	0.19
17	17x	isomenthone	31.7
18			0.15
19		x (ketone)	tr
20		(4-terpinenol)	1.0
21			1.7
22		(isopulegone)	3.2
23	21x	menthol	0.10
24		pulegone	59.86
25			0.14
26	26x	(borneol)	0.63
27			0.10

Parentheses indicate tentative identification. tr = trace.

Table 2. Per cent of total oil for four major monoterpene constituents in progressively older leaves of a stem of *H. drummondii*

Leaf No.	(+)-Limonene	Pulegone	Isopulegone	Isomenthone
2	8.8	84.7	nr	0.7
4	4.1	90.7	nr	0.2
6	1.6	92.2	nr	0.4
8	1.1	90.9	nr	0.3
10	1.1	92.5	0.4	1.2
12	0.3	79.5	2.4	9.8
14	0.2	28.0	2.5	63.4
15	0.2	09.9	1.5	78.4

Leaf No. 2 is nearest the bud tip. nr = not registered.

seems to indicate that the biosynthetic pathway of (+)-limonene in *Hedeoma* is certainly not terminal as they have suggested for other species [13, 14]. In *Hedeoma*, (+)-limonene must either be a part of a more involved pathway, or something must trigger a reversal of the pathway in order to explain the high concentration of the compound in very young tissue and its quick decrease as other compounds are produced. Such a scheme has been suggested by Murray and Lincoln [15].

The second major shift in the developmental sequence appears in the older leaf pairs (usually nodes 12–15 numbering downward). As these leaves become senescent and lose chlorophyll, pulegone and isomenthone go through a reciprocal change. This shift is similar to that found in *Mentha* [16] and supports the theory that pulegone may be produced through the reduction of piperitenone (rather than the oxidation of pulegol) and undergoes further reduction to form menthone or isomenthone. The shift of pulegone and isomenthone seems to correlate more with senescence than age. Occasionally a leaf will become senescent and undergo the chemical shifts described earlier than older leaves on the same stem. This increases the variation among leaves of the same node on different plants.

Both the stem and corolla were analysed and showed only a small amount of pulegone. In contrast, however, the calyx had a relatively high amount of oil with large numbers of oil glands being easily seen along its surface. The oil composition of the calyx was found to be generally like that of the leaves at the same node until senescence. At that time the pattern underwent a more dramatic change than it did in the leaf, with larger quantities of the reduced compounds appearing. A dramatic increase in compound #20 (tentatively 4-terpineol) occurred that was never seen in the aging leaves (Table 3).

As some monoterpenes are probably catabolized [17–19], it is not surprising that the reduction of certain compounds corresponded with senescence. At this period of development, respiration rates are reduced, and starch, chlorophyll, and protein are being catabolized and transported to other parts of the plant. As products are broken down and energy compounds formed, reducing conditions would be maintained, and the reduction of monoterpene compounds might initiate their catalysis.

Table 3. Per cent of total oil for major monoterpene constituents in two *H. drummondii* flowers of different age

Compound	Young flower (expanding)	Senescent flower
(+)-Limonene	2.61	1.21
Menthone	tr	1.36
Isomenthone	0.19	83.73
(4-Terpineol)	1.48	4.71
Isopulegone	1.99	1.42
Menthol	tr	1.02
Pulegone	89.25	1.24

The change in oil composition detected during maturation, when using whole plant steam distillation, is apparently due to the increased number of yellowing calyxes rather than leaves near senescence, which are few on a given plant. Since the calyxes are somewhat persistent, each plant accumulates 4–8 flowers at each node with perhaps only one or two being in the young green stage.

A statistical analysis indicated significant differences (α 0.05) between leaf pairs at different nodes for seven compounds (including 15, 17x, 19, 20, 24). For both pulegone and isomenthone, leaves from the fourteenth node were significantly different from each of the six younger pairs tested, emphasizing the dramatic and rapid change that occurs in senescing leaves. When the oil from the bud was added to the test, (+)-limonene was shown in significantly higher amounts there than in each of the other leaf pairs, and in one of the clones tested (Montana), the amount in leaves of the second node was significantly higher than each of the other nodes.

The results of this study indicate that some caution should be exercised in obtaining chemical data for systematic purposes. The induction technique can be useful but care needs to be taken to use leaves that are at the same stage of development. Leaves which are not at one end of the developmental sequence (i.e. very young or near senescence) should also be selected, as it is difficult to get consistent patterns for these leaves even on different branches of the same plant. In the case of *Hedeoma*, it would appear that leaves taken at the sixth through the ninth nodes are fairly representative of the total oil of the entire plant and are also quite consistent in pattern. Other work [20] has shown, however, that this may be somewhat dependent on the conditions under which the plant is grown.

While distillation of all, or most of the plant has a tendency to average the differences of leaves in different stages of development, the overall stage of plant development when collected must be considered. For *Hedeoma*, it is important to determine approximately how long the flowering process has proceeded, as the build up of flowers causes major shifts towards the reduced compounds.

Some of the taxonomic work that has been done using monoterpenes has been on a qualitative basis with only the presence or absence of a compound being considered rather than relative amounts. In many studies, however, a compound found in only small, or "trace" amounts, is considered absent. Our studies have shown that there are cases where the quantitative shift due to developmental changes is so great that some patterns could be considered qualitatively different even though the plant material is from the same clone and the compound is still present in very small amounts.

EXPERIMENTAL

Plant material. Clonal material of *Hedeoma drummondii* var. *drummondii* was grown by taking stem cuttings from a plant

collected at Big Timber, Montana and growing a plant from seed collected near Del Rio, Texas (Irving-#755). Cuttings were made at a point just below the fourth node down and were rooted, using Rootone, and grown in a potting mixture. The cuttings were grown in a growth chamber with illumination from cool white fluorescent and incandescent bulbs (24.84 klx). Twelve-hour light (temp. 26.7°) and dark (temp. 15.6°) periods were used.

Oil extraction and analysis. For steam distillation, the entire plant above soil level was excised and immediately placed in the distillation flask. After 2 hr of distillation, the ether fraction was removed, placed in glass vials, and evapd in a warm water bath. The oil fractions remaining in the vials were then sealed and placed in a freezer to await analysis. Syringe injections of 0.25 μl were made into the GLC. When using single leaf analysis, a leaf or a leaf pair was removed and weighed, placed in an indium capsule, and put in an inductor at 260°. As several pairs were harvested at the same time (*ca* 1 hr after the start of the light period), the capsules waiting for analysis were placed in a refrigerator at 4°. Previous work had shown that no detectable differences were created by this procedure [20]. GLC was done using an FID. The columns were 3.81 m × 3.175 mm and packed with 2.5% polyethelene glycol on chromosorb G. The temp. was linearly programmed 45–180° at 4°/min. The carrier gas was N_2 at 20 ml/min.

Statistical analysis. A computer program was used to check for proper calculation of the total number of microvolt counts/peak and to calculate the % of the total oil for each peak. Another program (SNK.F4), written by Robert P. Adams (Fort Collins), was used to perform an analysis of variance (ANOVA) for each compound and test for significant differences through use of the Student–Newman–Keuls multiple range test. Seven leaf pairs (every other one from node #2 downward) from each of four cuttings in each clone were analysed. In several cases it was necessary to consider several compounds as one character (Table 1) as there was not sufficient GLC separation to give separate integrator printouts. These groupings are designated with an '*x*'.

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