Changes in Essential Oil Quantity and Quality Influenced by Ontogenetic Factors

É. Németh
Department of Medicinal and Aromatic Plants
BKÁE, Faculty of Horticultural Sciences
Budapest, 1518. P.O. Box 53
Hungary

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Abstract

Connection between the developmental stage and the quantity and quality of essential oil was investigated in some model species of the *Asteraceae, Lamiaceae* and *Apiaceae* plant families.

The accumulation of essential oil decreased continuously during ontogenesis in *Tanacetum vulgare*. The tendency of accumulation showed an optimum curve in *Mentha piperita, Hyssopus officinalis, Majorana hortensis,* with a maximum bound to the flowering stage. However, the actual maximum may be influenced by ecological and agrotechnological factors. In the case of *Carum carvi* and *Foeniculum vulgare,* the accumulation tendency can be characterised by parallel optimum curves in vegetative and generative organs, with maximum points at different times and different levels.

The composition of essential oil of *Achillea* species during the leaf rosette stage was characterised by the abundant presence of sesquiterpenes, while after the generative differentiation the monoterpenes became dominant. In families *Lamiaceae* and *Apiaceae* the compositional changes are continuous not influencing determination of chemotypes. Alterations in composition of the essential oil may be based on structural changes of accumulation organelles or originating from modifications in biosynthesis or metabolization of synthesised products.

INTRODUCTION

Chemical diversity of essential oil producing species is a wellknown phenomenon. Recently, we have conducted a large series of investigations on the specialities and regularities of these chemotaxonomical changes both in quantitative and qualitative respects, focusing mainly on three families: *Asteraceae*, *Lamiaceae* and *Apiaceae*.

Oil Accumulation

Ruminska (1970) declared that essential oil content of *Asteraceae* species were highest at full flowering stage, however data for *Achillea* species and *T. vulgare* was not available. For peppermint, some authors found the highest value of essential oil content at the beginning of blossoming (Hornok, 1978; Clark and Menary, 1979), other authors found the essential oil content highest at full flowering time (Singh et al., 1995; Malizia et al., 1996). In fennel, Paukov et al. (1971) found a twenty-fold increase in essential oil content from budding till waxy ripening and Tóth (1967) reported the green-fruit stage as optimal for essential oil extraction. However, Cavaleiro et al. (1993) measured a double essential oil content in the ripen fruits compared to the green ones. Similarly, Gupta et al. (1995) mentioned the ripen fruit stage as assuring the highest level of essential oil.

Compositional Changes

In *A. millefolium* both Ruminska (1970) and Usztojzsanin et al. (1987) measured a maximum of chamazulene during early bud stage. Cernaj et al. (1983), however, determined the highest chamazulene level at full flowering. In the level of the studied four monoterpenes (α- and β-pinene, limonene, sabinene) alterations between the vegetative and generative phase proved to be significant, changes during flowering were slighter. Similar information in our model species (*A. pannonica*, *A. ochroleuca* and *A. crithmifolia*) were not known. In *Tanacetum vulgare*, Schantz et al. (1966) reported small quantitative changes (5-15%) of the main components of the essential oil from green bud stage till the end of flowering.

In peppermint and Chalchat et al. (1997) proved a continuous increase of menthol content in developing shoots. According Farooqi et al. (1999), after full flowering a decrease in menthol can be measured. In hyssop, Schulz and Stahl-Biskup (1991) found no significant compositional changes occur between vegetative and flowering shoots. On the contrary, Tsankova et al. (1993) reported the decrease of cineole (by 9%) and iso-piocamphone (by 12%) in the leaves during flowering.

During ontogenesis of fennel, Tóth (1967) and Trenkle (1972) found a decrease of anethole, fenchone and estragole in older shoots, while their proportion increased in the fruits. The tendencies for phellandrene and limonene are opposite. Cavaleiro et al. (1993) however, did not find significant differences in the proportion of these three major components during the fruit development.

MATERIAL AND METHODS

For the examination of accumulation dynamics and composition of essential oil, the model populations had been investigated at different phenological phases as follows.

Aboveground parts of *Tanacetum vulgare* (*Asteraceae*) chemotypes were sampled after sprouting out; at a stage of 30-40 cm vegetative shoots; at budding stage; at the beginning of flowering; at full flowering and at the seed ripening stage (Németh et al., 1994). Aboveground parts of *Achillea* species (*A. pannonica*, *ochroleuca* and *crithmifolia*) were cut at leaf-rosette stage; at 20-30cm height of the shoots; at budding stage and at full flowering (Zámboriné, 1996). The shoots of *Hyssopus officinalis* (*Lamiaceae*) were investigated from the vegetative stage after shooting through the seed ripening phase. Three populations (Hungarian cultivar ‘Kékvirágú’, gene bank materials of German origin, and a commercial material ‘Bulgarische Auslese’ were studied during two growing seasons (Németh et al., 2001). In *Mentha piperita* the cultivar ‘Mitcham’ was studied, sampling the flowering shoots during the generative phases from budding till the end of flowering (Zámboriné and Tétényi, 1988). In *Foeniculum vulgare* (*Apiaceae*) the vegetative parts and the generative parts were studied separately. During ontogenesis, the vegetative organs of cultivar ‘Soroksari’ had been sampled after shooting, before flowering and at full flowering. The generative parts were studied from the green bud stage through fruit ripening at nine different stages (Bernáth et al., 1999).

All of the samples were hydro-distilled according to the PhHgVII, and the main components of the oils were determined by capillary GC method (Bernáth et al., 1999).

RESULTS

Oil Accumulation

In *Tanacetum vulgare*, the essential oil content was the highest in the young shoots right after sprouting (0,66%). After this stage the oil content continuously decreased in the shoots and the minimum value (0,14%) was reached at seed ripening stage. The changes are parallel in each of the examined chemotypes.

In peppermint, the essential oil accumulated until the beginning of flowering (1,77%), and a significant decrease could be observed afterwards (1,41%), (Zámboriné and Tétényi, 1988). In hyssop, the essential oil level proved to be relatively low after sprouting, and it increased during the development of the shoots. The highest oil content was measured at full flowering, after which the oil content decreased again (Fig. 1). The tendency seems to be similar in each investigated taxa, with some smaller fluctuations during the flowering period.

In the vegetative organs of *Foeniculum vulgare*, a small peak in the roots, leaves and stems can be observed during flowering time (Table 1) (Chung and Németh, 1999).

In the generative organs, from the appearance of buds until the stage of green fruits, the *relative* percent of essential oil increased, then at ripening of the fruits, this trend returned (Bernáth et al., 1999).

Compositional Changes

In *T. vulgare*, a significant change of the main components of the essential oil was found during the examined phenological phases (Németh et al., 1994). The proportion of monoterpene components (borneole, camphor, 1,8-cineole, thujone and thujene-acetate) increased; while the level of sesquiterpene davanone decreased continuously (Table 2). All of the studied *Achillea* species exhibited a similar tendency. At the leaf-rosette stage the oil consists of mainly sesquiterpenes (Fig. 2). During the development of the shoots the rate of monoterpenes increased and at the time of full flowering, the main component was usually a monoterpene (Zámboriné, 1996).

In peppermint, the menthol content of the shoots in vegetative stage (44,1%) was significantly lower than the content after differentiation of the flowers (55,8%), (Zámboriné and Tétényi, 1988). In hyssop, pinocamphone increased and iso-pinocamphone decreased during shoot development (Table 3). Other major constituents of the oil (limonene, β-pinene) did not show significant changes (Varga et al., 1998).

Among the vegetative parts of *F. vulgare*, the largest compositional changes were detected in the roots. The proportion of anethole decreased by 37 percent, while the other phenyl-propane constituent, dillapiole, increased till 92 percent (Table 4). The proportion of the terpenoid components was permanently low. In the stems and leaves, the main component of the oil through ontogenesis was anethole, however its rate slightly decreased. In the leaves, there was also a small increase of fenchon and a slight of β-pinene (Chung and Németh, 1999). In the generative organs, anethole was the main essential oil compound, and both this and other major components showed a rather stable proportion (Table 5), (Bernáth et al., 1999).

DISCUSSION

It could be concluded that both the dynamics of accumulation of essential oil and the changes in its composition during ontogenesis are characteristic for each taxa. In the *Asteraceae* family, a continuous decreasing tendency was observed in shoots of *T. vulgare*, while a special optimum curve with a maximum bound to the flowering stage was detected in Lamiaceae species (*M. piperita* and *H. officinalis*). Our data is congruent with the results of Hornok (1978) and Clark and Menary (1979). It seems likely however, that the exact maximum point of the essential oil is influenced by several factors especially by the leaf/stem proportion, furthermore by the ecological and technological circumstances. Therefore, optimization of harvesting should be based on actual chemical analysis.

Oil accumulation of *F. vulgare* showed different optimum curves in vegetative and generative organs. It was found that the decrease of oil during fruit development is not an absolute decrease, but reflects a relation to other constituents of the fruits. The parallel rapid growth of non-fruit storage materials results in a decreasing relative value. Our results on *F. vulgare* are congruent with the findings of Szmoljanov and Kszendza (1976) and Bouwmeester (1998) who detected the similar trend of essential oil accumulation in the developing fruits of caraway, thus, it seems, that the described trends are characteristic for other *Apiaceae* species.

The compositional changes of mono- and sesquiterpenes in our *Asteraceae* model species, were linked to the developmental phases and appearance of generative organs. In *Lamiaceae* species, metabolization of synthesized compounds seems to be responsible for changes in oil composition. Concerning the contradictions in the literature, it may be concluded that ontogenetically determined changes of the composition are affected by other factors (weather, age and condition of plantation). In fennel, our results on changes of the main components are in harmony with the findings of Tóth (1967). In *Apiaceae* species the changing accumulation structures may contribute to compositional differences.

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Tables

Table 1. Changes of essential oil content in different organs of *Foeniculum vulgare* during ontogenesis.

Phenophase	Root	Stem	Leaf	Phenophase	Fruit
		(% of d.w.)			(% of d.w.)
Leaf-rosette	0,16	0,05	0,54	Green bud	1,12
Flowering	0,34	0,08	0,62	Yellow bud	2,20
Fruit ripening	0,27	0,06	0,41	Appearance of stamina	3,11
				Full flowering	4,64
				End of flowering	7,79
				Green fruits	11,59
				Waxy fruits	7,58
				Half ripen fruits	5,20
				Full ripening	6,61

Table 2. Accumulation of main component in six *Tanacetum vulgare* chemotypes during development in the apical (10-15 cm) shoot region (Németh et al., 1994).

Phenological phase	Chemotype					
	borneol	camphor	cineole	thujene-acetate	thujone	davanone
			(% of essential oil)			
Shoot of 10-15 cm	24	28	32	38	46	90
Shoot of 30-40 cm	22	26	34	42	50	79
Green bud phase	23	34	35	49	58	74
Full flowering	32	41	40	70	61	66
Seed ripening	30	28	31	33	62	51

Table 3. Changes in the proportion of main components in the shoots of *Hyssopus officinalis* during ontogenesis '*Kékvirágú*' Hungarian cultivar.

Phenological phase	β-pinene	limonene	pinocamphone	isopino-camphone
	(% of essential oil)			
Young shoots of 10-15 cm length	20	3	4	71
Vegetative shoots of 20-25 cm	18	2	2	74
Pudding	17	2	8	72
Start of flowering	19	2	6	69
Full flowering	17	3	9	67
End of flowering	14	2	15	64
Start of seed ripening	16	5	12	63
Seed ripening	14	5	14	63
Vegetative shoots at re-shooting	12	5	8	67
Second flowering	15	6	14	61

Table 4. Main components of essential oil of vegetative organs of *Foeniculum vulgare* at different phenological phases.

Compound	Root			Stem			Leaves		
	1	2	3	1	2	3	1	2	3
	(% of essential oil)								
α-pinene	tr	tr	tr	tr	tr	tr	0,7	tr	tr
β-pinene	tr	2,0	tr	tr	tr	0,4	5,9	0,8	tr
β-mircene	2,3	0,3	0,5	1,0	2,2	2,4	2,2	0,5	1,0
fenchone	3,5	0,7	0,8	1,0	tr	0,7	3,4	3,3	12,3
estragole	2,2	0,5	0,0	2,0	1,3	1,3	2,5	2,3	1,9
anetole	39,8	20,6	2,0	95,5	93,3	72,3	82,2	87,6	77,8
dillapiole	53,6	75,3	92,1	tr	1,3	39,0	0,0	0,8	tr

1- leaf-rosette stage; 2- flowering stage; 3- waxy ripening stage; tr=trace

Table 5. Main components of essential oil of generative organs of *Foeniculum vugare* at different phenological phases.

Phase	α-pinene	β-pinene	mircene	limonene	fenchon	methyl-cavicol	anethole
	(% of essential oil)						
Green bud	3,0	1,3	7,3	3,5	9,1	3,4	64,5
Yellow bud	3,0	1,1	4,0	3,2	16,4	2,4	65,3
Appearance of stamina	3,9	1,5	3,4	3,4	16,8	2,4	63,8
Full flowering	4,0	1,6	2,5	3,5	20,9	3,2	60,4
End of flowering	5,0	2,0	1,6	3,8	25,3	2,8	55,5
Green fruits	4,7	2,0	0,9	4,4	27,9	4,8	53,4
Waxy fruits	2,6	1,3	ny	2,5	24,7	2,4	65,4
Half ripen fruits	2,7	1,3	ny	2,7	21,4	2,6	68,4
Full ripening	3,3	1,7	ny	3,1	23,2	2,5	65,3

Figures

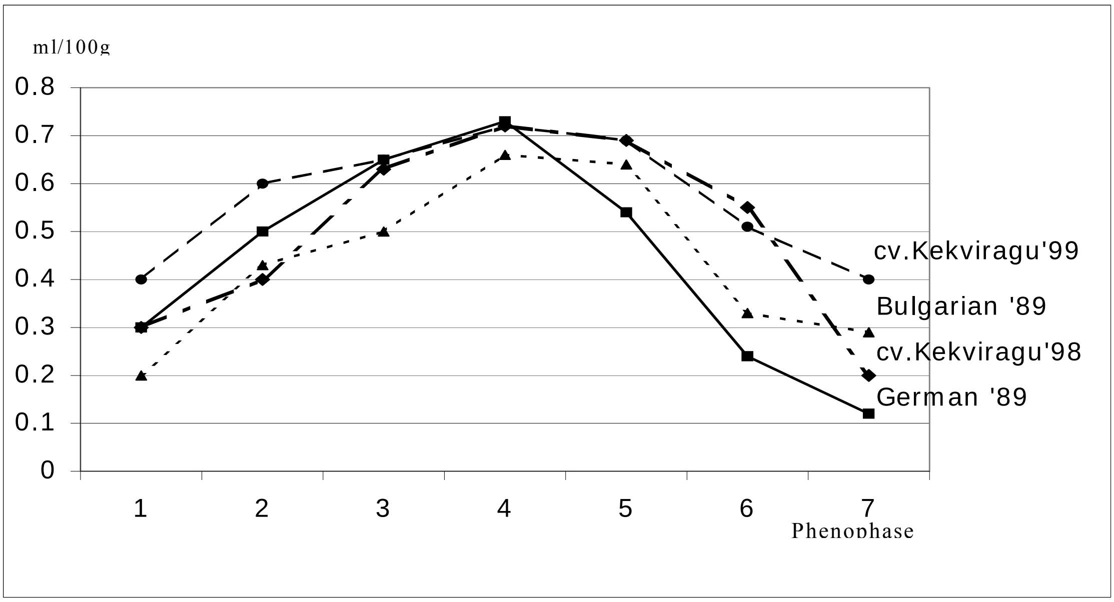

Fig. 1. Essential oil content of the shoots of *Hyssopus officinalis* during ontogenesis. 1: after shooting; 2: vegetative shoots; 3: budding shoots; 4: start of flowering; 5: full flowering; 6: end of flowering; 7: seed ripening.

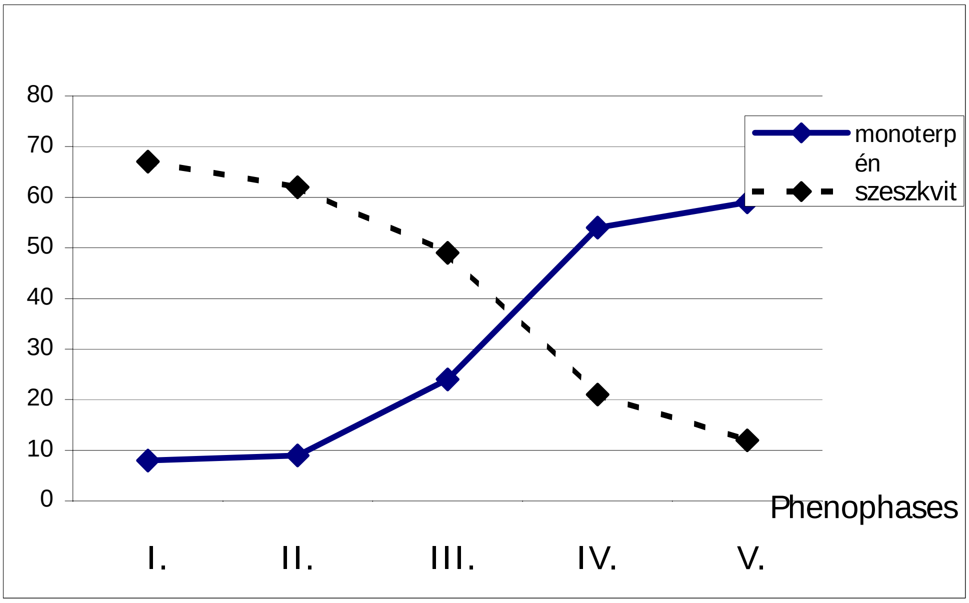

Fig. 2. Changes of the main terpenoid fractions in the essential oil of *Achillea pannonica* during ontogenesis. I. leaf rosette stage; II. start of shoot growth; III. vegetative shoots; IV. budding stage; V. full flowering.