

Variation of Monoterpenoids in *Artemisia feddei* and *Artemisia scoparia*

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The composition and concentration of monoterpenoids in the leaves and stems of *Artemisia feddei* and *Artemisia scoparia* were determined, and seasonal variation in the monoterpenoids of *Artemisia* species were investigated. The two species possessed different compositions and concentrations of monoterpenoids. The total amount of monoterpenoid in *A. scoparia* was always higher than that of *A. feddei*, and the monoterpenoid yields of leaves were higher than stem yields in both species as compounds formed. The major constituents of *A. scoparia* were 25 while *A. feddei* consisted of 26 compounds. Sixteen common monoterpenoid compounds were found in both plants. Large differences in the relative amounts of the monoterpenoids were found between species and seasons. Extremely large differences in the relative amounts of naphthalene, sabinene, β -pinene, cyclohexene, and octatriene were found in the leaf monoterpenoids of the two species. The largest differences in relative amounts of stem monoterpenoids were in sabinene and β -pinene levels.

Keywords: monoterpenoid, *Artemisia scoparia*, *Artemisia feddei*, Sabinene, β -pinene

Among all of the terpenoids, mixtures of volatile monoterpenoids and sesquiterpenoids (called essential oils, which are found in high levels in plants) are being increasingly implicated in plant-pest interactions (Edwards *et al.*, 1993). Essential oils have well-known insect repellent properties apart from their commercial value. Many monoterpenoids and their derivatives are especially important as agents of insect toxicity (Mattson *et al.*, 1988; Croteau, 1981) and allelochemicals (Kil *et al.*, 1991).

Terpenoids play an important role in the complex interactions within the ecosystem. Mixtures of volatile monoterpenoids lend a characteristic odor to plant foliage. Plant terpenoids have been widely used in taxonomic (Williams *et al.*, 1995), phylogenic (Harbone and Turner, 1984), microbial (White, 1986) and ecological (Langenheim, 1994; Kim and Langenheim, 1994) studies.

Beginning in the late 1960s, many secondary products were shown to have important ecological functions in plants (Harbone, 1982). Among these chief functions is protection against herbivore and infection by microbes. More important terpenoids have been shown to serve as attractants for pollinators and fruit-

dispersing animals and as agents of plant-plant competition.

The genus *Artemisia* has been the object of numerous chemical studies (Marco *et al.*, 1994a, 1994b; Yasphe *et al.*, 1987; Kim, 1996; Kil *et al.*, 1991). There are large amounts of monoterpenoids and their derivatives in *Artemisia* species (Ahmad and Misra, 1994). Large variations in the amounts of the constituents were found to due to plant age (Langenheim *et al.*, 1986), season (Nerg *et al.*, 1994), and in comparison of tissues of individuals (Kristina *et al.*, 1996). A previous study of *A. princeps* var. *orientalis* (Kim, 1996) indicated that there were marked qualitative and quantitative differences between plant parts and seasons, the monoterpenoid yields were more variable than monoterpenoid compositions in terms of seasonal variation.

Artemisia plants are widespread. 26 species occur in Korea. Most *Artemisia* plants have been used in traditional biomedicine for intestinal bacteria, as food, and for many other purposes in Korea. In spite of this, little is known about the amount or constituents of seasonal variation within and between the *Artemisia* species in Korea. Li *et al.* (1995) reported the phenotypic and ontogenetic variations in volatile leaf oils between and within the 12 species from the informal subgenus *Monocalytrus* in Tasmania. Many

studies (Espinosa *et al.*, 1991; Rafii *et al.*, 1996; Barton *et al.*, 1991) have suggested that volatile terpenoids have been used as chemical indicators to classify terpenoid containing plants. The objective of this study is to identify and quantify monoterpenoids, to evaluate seasonal variation, and to find the interspecific differences of monoterpenoids between *A. scoparia* and *A. feddei*.

MATERIALS AND METHODS

A. scoparia and *A. feddei* were collected from five different sites at Mt. Muhak during their maturing periods at approximately one week intervals. Samples were sealed in plastic bags and transported to the laboratory. Plants were separated into leaf and stem, and three grams of subsamples were immediately ground with pure sand and extracted with n-pentane and one ml, 1% tetradecane as an internal standard. The pentanes were steam distilled three times, using a glass distillation unit for 6 hours, which was used for all chemical analyses to increase the purification. Plant extracts were filtered with sodium sulfate and concentrated by evaporation with a gentle stream of nitrogen gas.

Samples were analysed by gas chromatography-mass spectrometry (Hewlett Packard 5890) using a 30 m long HP5 (i.d. 0.25 mm, a flame ionization detector) capillary column. Helium gas was used as the carrier gas. The temperature program for terpenoids was initially 37°C for five minutes, increased to 180°C at a rate of 5°C min⁻¹, then by 20°C min⁻¹ until 320°C was reached.

Individual terpenoids were identified by their mass spectra and retention times. Peak areas were used to quantify the individual substances. For quantification, calibrations were made, using known amounts of available pure terpenoids, and response factors were determined for each substance relative to known amounts of the internal standard. The concentrations of some monoterpenoids were not identified because there was no available standard and the chromatograms were too complex. In this study, although some monoterpenoids were not present in quantities large enough to identify, the yield of absolute concentrations, the relative differences, and the assessment of seasonal variation in the two species are valid.

The ANOVA for variation in monoterpenoid components and t-testing for the differences of terpenoid amounts between two species were computed using the Excel program (ver. 7.0).

RESULTS

Approximately 35 monoterpenoids and other compounds were detected in *A. scoparia* and *A. feddei*. However many compounds were present only in small or trace amounts. Fig. 1 and Fig. 2 show a gas chro-

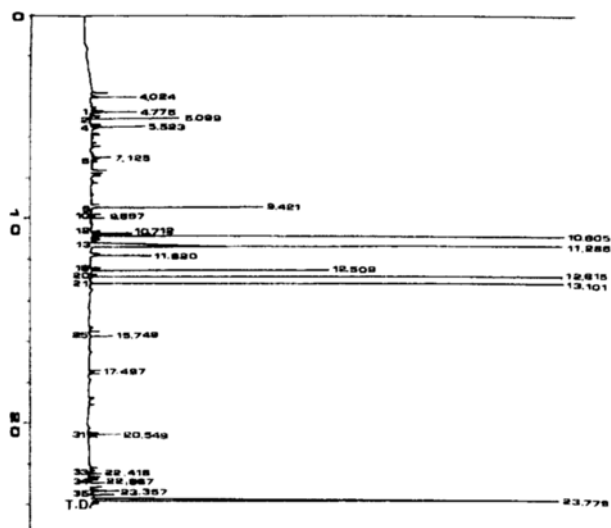


Fig. 1. Gas chromatographic assesment of the extraction from *Artemisia scoparia* leaf in June. Many monoterpenoids are presented only in small or trace amounts. Number means monoterpenoid compound with the same No. in Table 1. T.D. means tetra decane as internal standard.

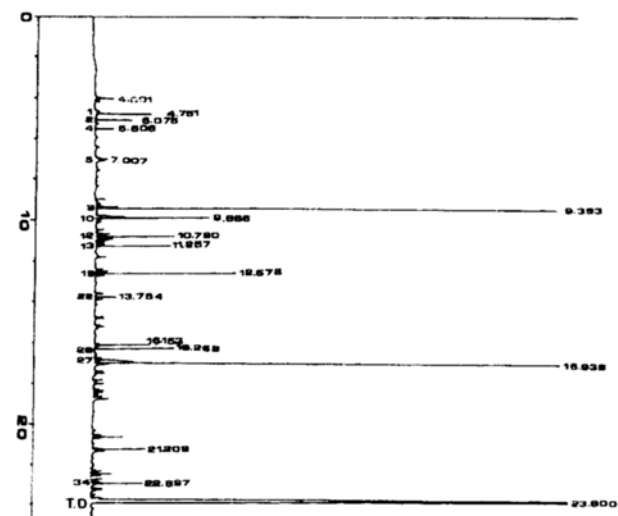


Fig. 2. Gas chromatographic assesment of the extraction from *Artemisia feddei* leaf in June. Many monoterpenoids are presented only in small or trace amounts. Number means monoterpenoid compound with the same No. in Table 1. T.D. means tetra decane as internal standard.

Table 1. The major monoterpenoids (%) in the leaf and stem of *A. scoparia* and *A. feddei*

Compound	Name	R.T.	<i>A. scoparia</i>		<i>A. feddei</i>		t-testing	
			Leaf	Stem	Leaf	Stem	Leaf	Stem
1	octene	4.73~4.64	3.96	6.63	13.2	25.58		
2		5.07~5.09	0.48	0.76	3.5	13.07		
3		5.34~5.37	3.59	0	0	0		
4		5.52~5.58	2.06	5.25	10.98	0.63		
5		7.12~7.18	0.34	0.13	0.91	5.43		
6	α -thujene	7.40~7.43	0.19	0.47	0	0		
7		8.01~8.04	0.06	0.16	0	0		
8		9.25~9.27	0	0	0.13	0		
9	α -pinene	9.41~9.45	3.67	5.03	8.72	2.16	*	
10	camphene	9.86~9.97	0.21	0.63	2.58	0.83		
11		10.52~10.56	0.65	0.32	0	0		
12	sabinene	10.76~10.79	21.59	23.69	3.41	1.75	**	**
13	β -pinene	11.24~11.27	20.5	21.48	3.92	2.49	**	***
14	β -myrcene	11.39~11.42	0	0.13	3.78	1.41		
15		11.89~11.93	0	0	0.91	3.3		
16		11.94~11.98	1.13	5.32	0	0		
17		12.19~12.22	0	0	0.38	0.08		
18		12.61~12.62	5.01	13.29	3.76	3.87		*
19	dl-limonene	12.58~12.59	0	0	6	1.56		
20	cyclohexene	12.92~12.93	19.78	13.35	1.82	0.54	***	
21	octatriene	13.26~13.27	9.42	0.14	1.16	0.46		*
22		13.62~13.64	0.25	0.23	0.23	0		
23		14.93~14.94	0	0	3.47	3.44		
24		15.32~15.34	0	0	1.25	1.19		
25	alloccimene	15.86~15.87	0.83	0.47	0	0		
26		16.26~16.28	0	0	2.38	2.35		
27	naphtalene	16.96~16.97	0	0	14.59	6.17	*	*
28	isomenthol	17.62~17.63	0.85	0.03	3.37	4.81		
29		18.77~18.78	0.22	0	0	0		
30		19.05~19.06	0.08	0.58	0	0		
31		19.18~19.19	1.25	0	0	0		
32	bornyl acetate	20.66~20.68	0	0	4.22	7.12		
33		22.41~22.58	0.31	0.73	0.25	2.52		
34	geranyl acetate	22.86~22.88	3.22	0.98	1.38	7.84		
35		23.35~23.40	3.34	0.42	3.7	1.41		
total			25	22	26	24		

R.T.: Retention Time

*: $p < 0.01$, **: $p < 0.001$, ***: $p < 0.001$

matographic assessment of the extraction from the levels of *A. scoparia* and *A. feddei*. There are differences in the monoterpenoid fractions of the two species. Twenty five monoterpenoids in *A. scoparia* and 26 monoterpenoids in *A. feddei* were analysed, and 16 common monoterpenoids were found (Table 1). The leaf monoterpenoid fraction of *A. scoparia* was dominated by sabinene, β -pinene, No. 18 compound, cyclohexene, and octatriene, which were detected in average amounts of more than 5% of the total monoterpenoids. The major relative amounts of *A. scoparia* leaf monoterpenoids ranged from 5.01 (No. 18) to 21.59% (sabinene), which were remarkable

differences compared to the concentrations of other compounds. The major constituents of *A. feddei* monoterpenoids were octene, No. 4 (R.T.=5.52), α -pinene, and naphtalene. They ranged from 8.72 (α -pinene) to 14.59% (naphtalene).

Fig. 3 shows the total concentrations of leaf monoterpenoids between *A. scoparia* and *A. feddei*. The total amounts of *A. scoparia* leaf monoterpenoids were always higher than those of *A. feddei*. The total amount of monoterpenoids ranged from 3.243 mg/g to 0.721 mg/g in *A. scoparia*, and from 1.31 mg/g to 0.298 mg/g in *A. feddei*. The total amount of both leaf and stem monoterpenoids in both species

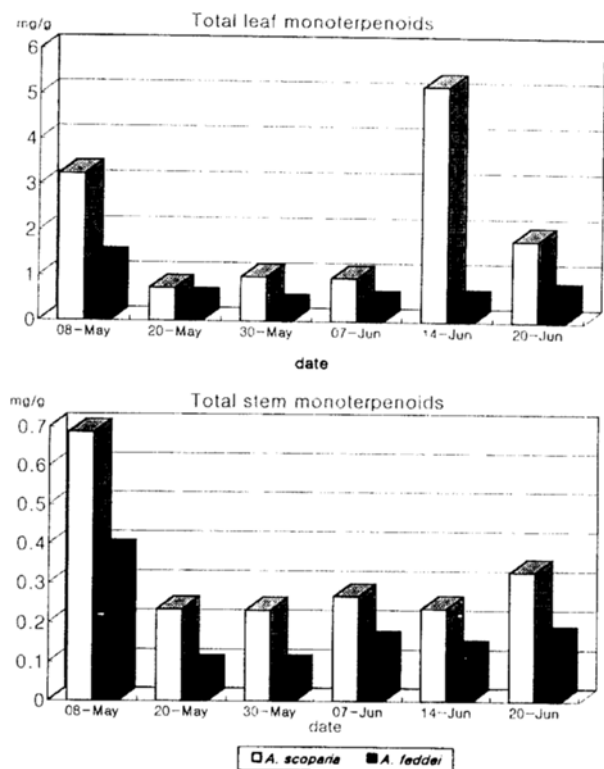


Fig. 3. Seasonal variation of total monoterpenoid concentration in *A. scoparia* and *A. feddei*.

rapidly decreased over time except for the *A. scoparia* leaf monoterpenoids of 14 June. After 29 May, the total amounts of monoterpenoids was almost constant in both species.

The total leaf monoterpenoid levels in *A. scoparia* ranged from 1.46 to 21.98 times the stem monoterpenoid levels in *A. scoparia*. The total amount of leaf monoterpenoids in *A. feddei* ranged from 2.71 to 3.69 times stem amounts.

The largest differences in relative amounts of leaf monoterpenoids between the two species were found in sabinene ($p < 0.001$), α -pinene ($p < 0.01$), β -pinene ($p < 0.001$), cyclohexene ($p < 0.0001$), octatriene ($p < 0.05$), and naphthalene ($p < 0.05$) (Fig. 4). The ratios of α -pinene and naphthalene in *A. feddei* were significantly higher than in *A. scoparia*. These compounds are the major monoterpenoids in *A. feddei*. Also, large differences in the relative amounts of stem monoterpenoids in the two species were found in levels of β -pinene ($p < 0.0001$), sabinene ($p < 0.001$), compound No. 18 (R.T.=12.61) ($p < 0.01$), cyclohexene ($p < 0.05$) and octatriene ($p < 0.01$) (Fig. 5). There are significant differences in the leaf and stem monoterpenoids of each species (Fig. 4 and Fig. 5), and the seasonal variation of the relative amounts of leaf and stem

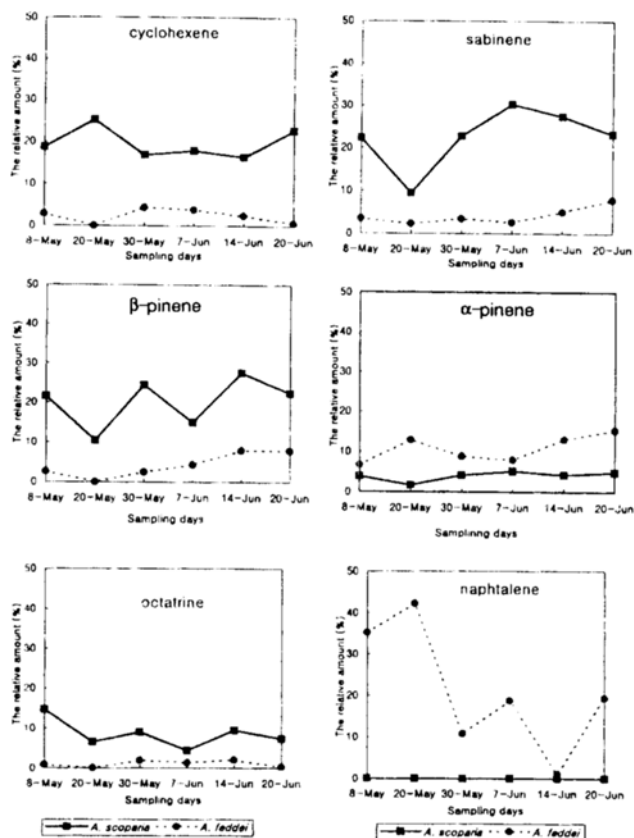


Fig. 4. The relative amounts of cyclohexene ($p < 0.0001$), sabinene ($p < 0.001$), β -pinene ($p < 0.001$), α -pinene ($p < 0.01$), octatriene ($p < 0.05$) and naphthalene ($p < 0.05$) in the leaf of *A. scoparia* and *A. feddei*.

monoterpenoid varied in both species with the exception of α -pinene and octatriene in the *A. scoparia* leaf.

Fig. 6 shows the seasonal variation of each monoterpenoid concentration in the leaf and stem of *A. scoparia*. The concentration of each monoterpenoid is represented over time. There were marked differences between each monoterpenoid, but the major components of leaf monoterpenoids were not varied ($p > 0.05$) in response to time. On the other hand, although the concentrations of stem monoterpenoids were detected in small or trace, there was much variation within constituents and across time ($p < 0.05$).

Fig. 7 shows the ratio of compositional monoterpenoid of the leaf and stem of *A. scoparia* over the time. There were no significant differences in the ratio of composition of monoterpenoids between leaf and stem of *A. scoparia*. Compared to these results from *A. scoparia*, there were highly significant differences in leaf ($p < 0.0005$), and stem ($p < 0.0001$) concentrations of monoterpenoids in *A. feddei* (Fig. 8). Contrary to *A.*

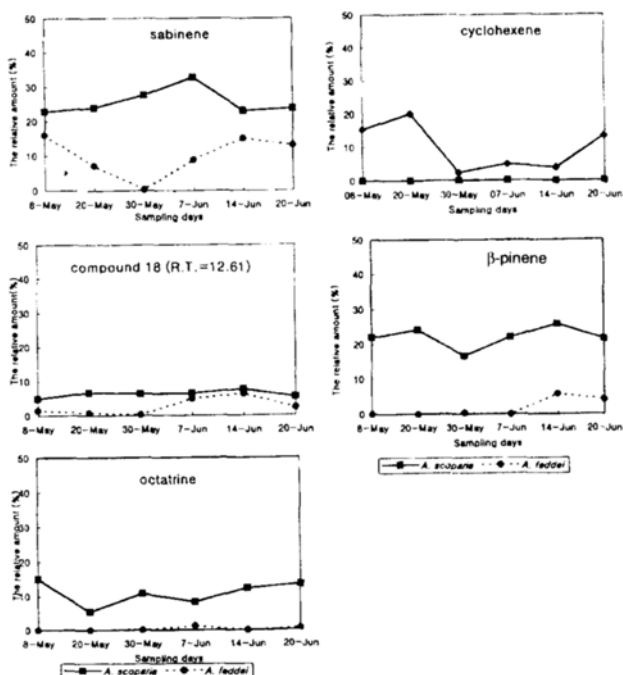


Fig. 5. The relative amounts of sabinene ($p < 0.001$), cyclohexane ($p < 0.05$), No. 18 compound ($p < 0.01$), β -pinene ($p < 0.0001$) and octatrine ($p < 0.01$) in the stem of *A. scoparia* and *A. feddei*.

scoparia, there were significant differences in the ratio of composition of monoterpenoids between leaf and stem of *A. feddei* ($p < 0.00001$) (Fig. 9).

DISCUSSION

There were many differences in the gas chromatography of *A. scoparia* and *A. feddei* (Fig. 1 and 2). Generally, we can smell the difference in aroma from the leaves of *A. scoparia* and *A. feddei* easily, and we can readily see the differences in the shape of young leaves of the two species. Certain plants contain mixtures of volatile monoterpenoids and sesquiterpenoids which lend a characteristic odor to their foliage. Peppermint, lemon, basil and sage are examples of plants containing such essential oils. The chief monoterpenoid constituent of peppermint oil is menthol, of lemon oil limonene, and of pine oil pinene. Therefore, many volatile monoterpenoid compounds may be involved in the olfaction of *Artemisia* species, with the major monoterpenoids having the high peak area. The high concentration of the monoterpenoids sabinene, β -pinene, and cyclohexene might give a characteristic odor to *A. scoparia*, while naphthalene might give a characteristic odor to *A. feddei*. Although the specific aroma of plants is repre-

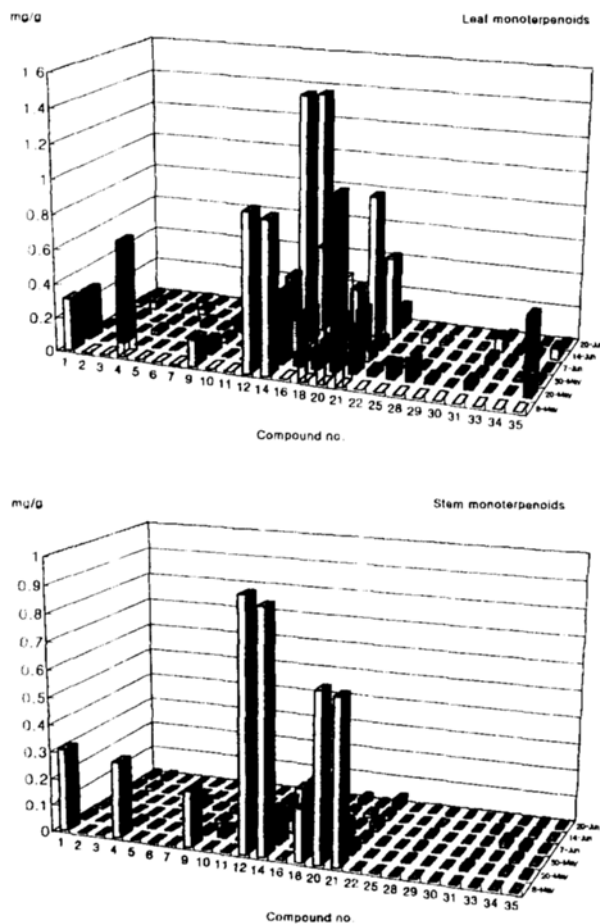


Fig. 6. Seasonal variation of each monoterpenoid concentration in the leaf and stem of *A. scoparia*. The concentration of each monoterpenoid varied in leaf ($p < 0.05$) and stem over time ($p < 0.05$).

sented by the highest concentration of constituents, we don't know which compound serves to give foliage its odor, because the compounds are composed of a mixture of several kinds of terpenoids.

Fig. 3 shows the total concentrations of leaf monoterpenoids in *A. scoparia* and *A. feddei*. The total amounts of *A. scoparia* leaf monoterpenoids were always higher than those of *A. feddei*. The total monoterpenoid levels were higher in early spring in both species. The reason for these results might be the differences in the shape of the young leaves of the two species. Because the shape of *A. scoparia*'s young leaf is a rosette and bunch type, the volatile monoterpenoid on the leaf might therefore be less volatile than on the expanded *A. feddei* leaf.

The total amounts of both leaf and stem monoterpenoids in the two species rapidly decreased across time with the exception of the *A. scoparia* leaf mo-

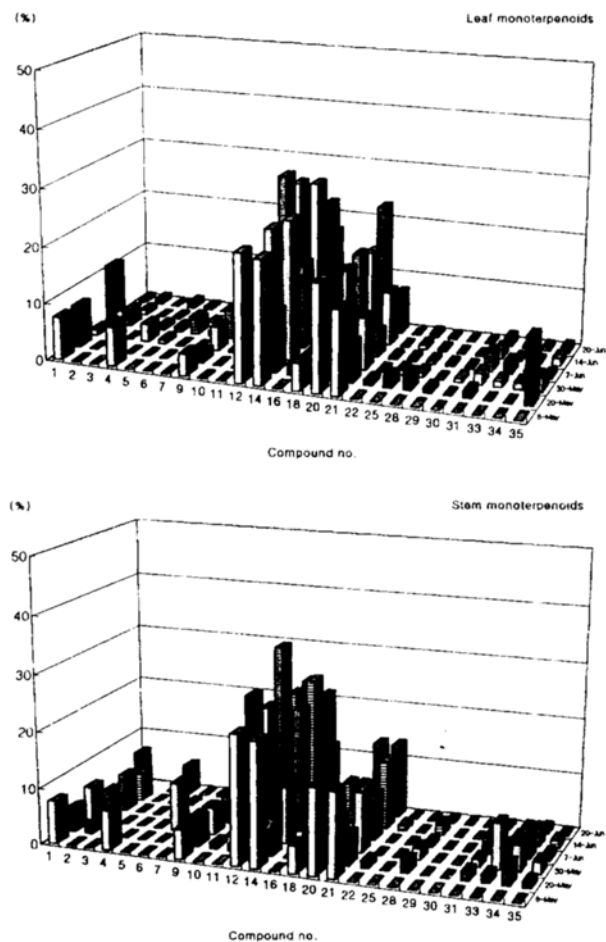


Fig. 7. Seasonal variation of compositional ratio of each monoterpene in the leaf and stem of *A. scoparia*. There are no significant difference in the ratio of monoterpenoids both in leaf and stem ($p > 0.1$).

noterpenoids of 14 June. After 30 May, the total amounts of monoterpenoids were almost constant in both species. These results represent the same trends shown by the leaf and stem monoterpenoids of *A. princeps* var. *orientalis* (Kim, 1996). In general, volatile terpenoids are effective deterrents because they repel herbivores prior to defoliation (Levin, 1976). Since the early stages of most plants are the most vulnerable to browsing by herbivores, Levin (1976) and Goralka (1996) predicted that increased levels of defensive compounds occur in seedlings (Goralka, 1996). In particular, the high concentrations of monoterpenoids in the early stages of *Artemisia* plant development might be advantageous for developing mechanisms to deter herbivores. Therefore, the high concentration of leaf monoterpenoids in *A. scoparia*, sabinene, β -pinene, and cyclohexene, seem to affect the activities of herbivores. The most distinctive fluctua-

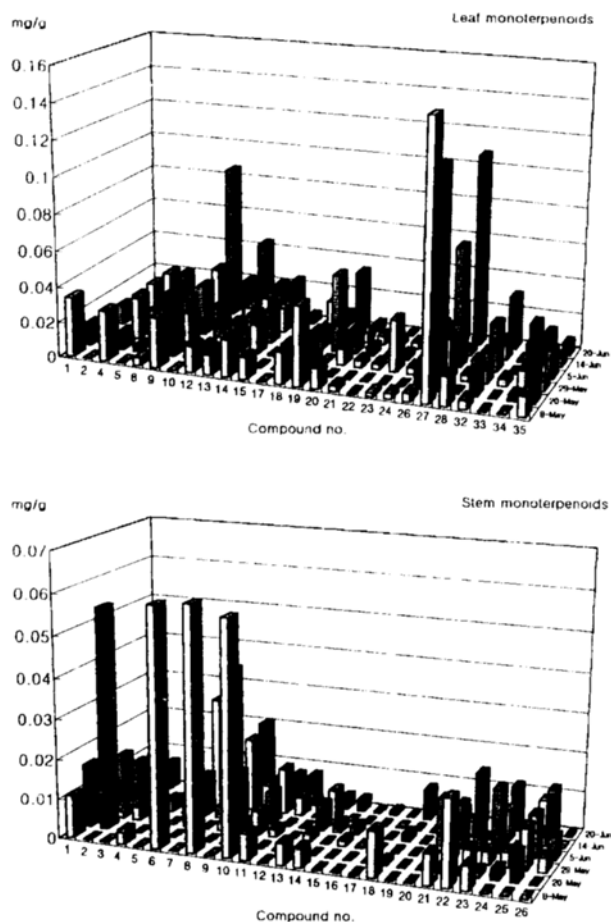


Fig. 8. Seasonal variation of each monoterpene concentration in the leaf and stem of *A. feddei*. The concentration of each monoterpene varied in leaf ($p < 0.0001$) and stem ($p < 0.0001$) with the time.

tations in monoterpene concentrations are normally observed during the development of pine needles (Rudloff, 1975; Hanover, 1966; Michelozzi *et al.*, 1990), and this was also observed in this present study.

The leaf and stem monoterpene fraction of *A. scoparia* was dominated by sabinene, α -pinene, octene, No. 4, No. 18 compound, cyclohexene, and octatriene which were detected in average amounts of more than 5%. The major leaf and stem monoterpenoids in *A. feddei* were octene, No. 4, α -pinene and naphthalene. This is markedly different from the levels of the other compounds, which were detected in average amounts of more than 5% (Table 1). Monoterpene composition is quite stable in monoterpene-containing species, but the absolute amount of individual and total monoterpenoids fluctuates during the seasons (Schonwitz *et al.*, 1990; Kim, 1996).

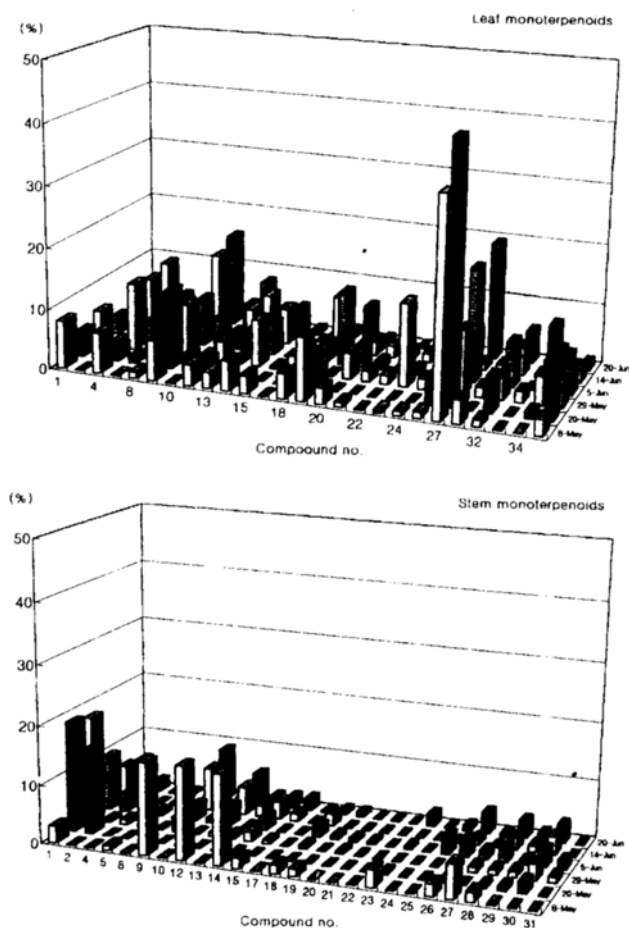


Fig. 9. Seasonal variation of compositional ratio of each monoterpene in the leaf and stem of *A. feddei*. The compositional ratio of each monoterpene were varied between leaf and stem ($p < 0.00001$).

This phenomenon was also found in the present study, in which the greatest differences were found in the leaf concentrations of sabinene, cyclohexene, α -pinene, β -pinene, octatriene, naphthalene and in the total monoterpene levels between species. Of these, α -pinene and octatriene were not varied with time. Nerg *et al.* (1994) and Kristina *et al.* (1996) reported that the differences in monoterpene composition between seasons and tissues are large but mainly quantitative. In this study some monoterpene concentrations and compositions of *A. feddei* were similar to *A. princeps* var. *orientalis*, those of *A. scoparia* were different. In *Artemisia* foliage, the concentrations of sabinene and naphthalene might be the primary monoterpenoids that determine chemotype. In the present study the absolute concentration of each monoterpene was varied across time, but the ratios of each monoterpene were not varied in *A.*

scoparia (Fig. 6 and 7). This may indicate that the mechanisms of monoterpene production is to some extent connected with the genetics of monoterpenoids. The amounts of monoterpenoids are strongly dependent on the amounts of structurally similar monoterpenoids. Kristina *et al.* (1996) found that the amounts of γ -terpinene and terpinolene, among other constituents, were strongly dependent on the amount 3-carene. Several researchers (Tobolski and Hanover, 1971; Zavarin *et al.*, 1990) have suggested that concentrations of monoterpenoids are regulated in part by biological mechanisms common to developing foliage.

As has been pointed out by many studies (Baradat and Yazdani, 1988; Nerg *et al.*, 1994; Yazdani and Lebreton, 1991; Barton *et al.*, 1991; Marco *et al.*, 1994), because the total proportional quantities of monoterpenoids are dependent on the species, monoterpene analyses between species and within species give increased information for chemotaxonomical studies. Such analyses also provide a new tool for investigation of biosynthetic routes.

ACKNOWLEDGEMENTS

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