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Regional and habitat differences in 7-methyljuglone content of Finnish *Drosera rotundifolia*

Terttu Kämäräinen[a,*], Jouko Uusitalo[b], Jorma Jalonen[b], Kari Laine[a], Anja Hohtola[a]

[a]*Department of Biology/Botany, University of Oulu, PO Box 3000, FIN-90014 Oulu, Finland*
[b]*Department of Chemistry, University of Oulu, PO Box 3000, FIN-90014 Oulu, Finland*

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Abstract

The concentration of 7-methyljuglone was studied in the round-leaved sundew *Drosera rotundifolia* L. collected from different regions in Northern Finland. Samples for analysis were collected from peat bogs and sandpit habitats. The mean concentration of 7-methyljuglone varied from 1.0 to 2.3% of dry weight. Variation between years in the amount of 7-methyljuglone was significant in plants growing on sand, and in the northernmost region studied. Overall, the variation in the production of 7-methyljuglone among different populations of round-leaved sundew in Northern Finland was rather low. The variation between years in the production of 7-methyljuglone was more significant.

Keywords: Round-leaved sundew; *Drosera rotundifolia* L.; Droseraceae; Naphthoquinones; 7-Methyljuglone; Secondary metabolite production

1. Introduction

Round-leaved sundew (*Drosera rotundifolia* L.) inhabits nutrient-poor, moist and sunny areas such as peat bogs and abandoned sandpits. *D. rotundifolia* is carnivorous gaining its nutrients, especially nitrogen and phosphorous from captured insects. It produces naphthoquinones, among other compounds, as secondary metabolites. The major naphthoquinones found in the Droseraceae family are plumbagin (2-methyl-5-hydroxy-1,4-naphthoquinone) and 7-methyljuglone (5-hydroxy-7-methyl-1,4-naphthoquinone). They also contain glucosides of the free quinones (Budzianowski, 1995, 1996; Schölly and Kapetanidis, 1989). These phenol compounds have pharmacological activity (Didry et al., 1998) and therefore sundews are used in medicinal preparations (Wurm et al., 1984). Several *Drosera* species are included in pharmacopoeias and dried plants are marketed as Herba Droserae, Herba Drosera rotundifoliae, etc. (Länger and Kopp, 1995).

The amount of naphthoquinones varies between different *Drosera* species (Bonnet et al., 1984) and in different tissues of the plants (Hook et al., 1997; Repčák et al., 2000). In some sundew species, the concentration of naphthoquinones varies during the growing season, but in *D. rotundifolia* the amount is fairly constant (Caniato et al., 1989). In *D. spathulata* the amount of naphthoquinones increases with increased level of differentiation during organogenesis (in vitro) and the composition of the growth media also has an effect on the production of naphthoquinones (Blehová et al., 1995).

The use of *D. rotundifolia* in medicinal preparations has been to some extent replaced by imported other species (*Drosera peltata* Sm., *Drosera madagascariensis* DC.) not native to Europe (Krenn et al., 1995). Although *D. madagascariensis* is poor in active compounds, it has been accepted in pharmacopoeias. The sundews are endangered in many countries and they are protected for example in Belgium (Leclercq and Angenot, 1984). The round-leaved sundew is presently not endangered in Finland (Hämet-Ahti et al., 1998), but the small size of plants makes collection from natural stands laborious and therefore, cultivation possibilities have been studied (Galambosi et al., 1999).

* Corresponding author. Tel.: +358-8553-1544; fax +358-8553-1500.
E-mail address: terttu.kamarainen@oulu.fi (T. Kämäräinen).

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To our knowledge the naphthoquinone production of *D. rotundifolia* at different latitudes has not yet been thoroughly studied. There are some indications that photoperiod and temperature can affect secondary metabolite production (Voirin et al., 1990; Jensen et al., 1995). The results presented in this paper are in most cases based on data obtained from analysis of several years of survey. The aim of this work was to examine concentrations of 7-methyljuglone of the round-leaved sundew in regions from different latitudes in Northern Finland. Another aim was to search populations with a high capacity for secondary metabolite production that would be suitable for cultivation.

2. Results and discussion

The concentration of 7-methyljuglone in different populations and different years of analysis varied from 1.0 to 2.3% of dry weight (Fig. 1). The mean concentration was 1.5%. There was about a fivefold difference between maximum (2.7%) and minimum (0.5%) concentration of 7-methyljuglone in individual samples from the different test areas (data not shown). The highest values, over 2.5% of dry weight, were found in samples from eutrophic *Carex* bog shore, sand substrate and from the northernmost sphagnum bog (populations three, five and nine, respectively).

There were statistically significant differencies in the production of 7-methyljuglone between years in the two sundew populations growing on sand substrate (populations two and five, Table 1) and also in the northernmost population from sphagnum substrate (population nine, Table 2). In rest of the populations the variation between years was quite small. There were big differences in the

Table 1
Results of the statistical analysis in variation in the production of 7-methyljuglone in populations 1–6

Population	ANOVA		Bonferroni		
	F	Sig.	Years	Mean difference	Sig.
1	2.524	0.114			
2	11.608	0.001	1 and 3	−0.438	0.001
3	1.757	0.218			
4	0.336	0.719			
5	16.111	0.001	1 and 2	1.196	0.001
			2 and 3	−0.730	0.014
6	1.718	0.228			

Results of 3 years comparisons (ANOVA, Bonferroni-tests). Only significant differencies are shown in Bonferroni-test results.

Table 2
Results of statistical analysis (*t*-tests) in variation in the production of 7-methyljuglone in populations 7–9

Population	Years	*t*	Sig.
7	1 and 2	0.361	0.724
8	1 and 3	−1.997	0.077
9	1 and 3	−3.336	0.010

Comparison between 2 years.

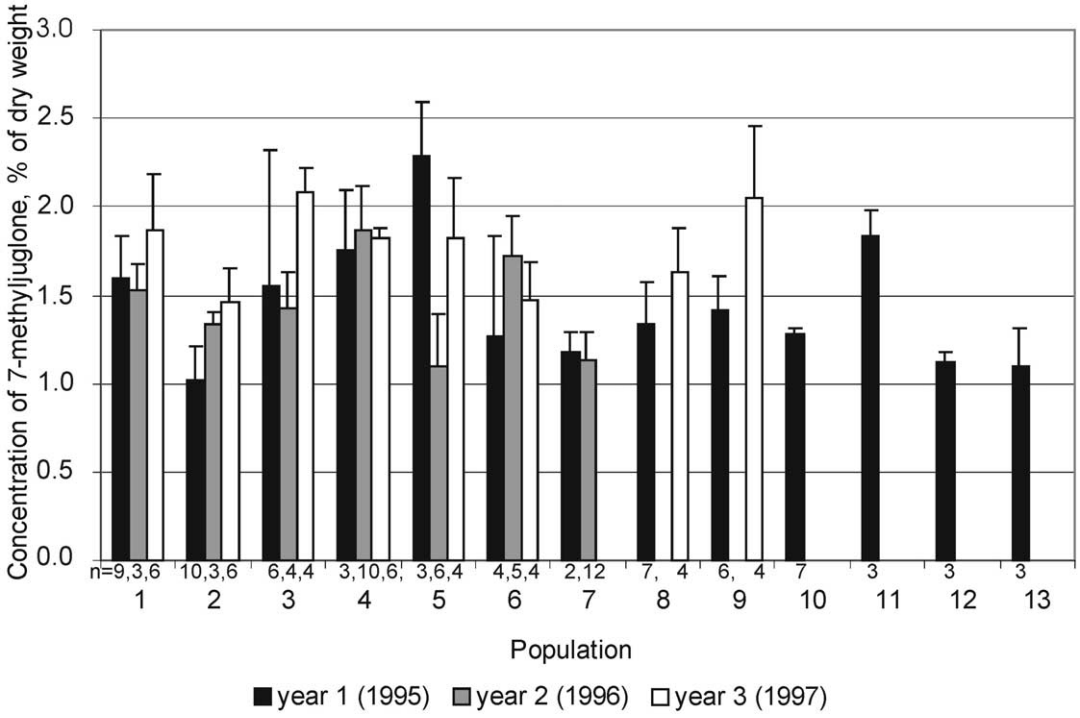

Fig. 1. Concentration of 7-methyljuglone in *Drosera rotundifolia* from different populations. Data represents mean concentration of 7-methyljuglone in the samples ± S.D., 1 × GC run per sample. *n* = number of replicates analyzed.

concentration of 7-methyljuglone between different samples in the same year, for example, in year one in populations number three and five (standard deviations in Fig. 1). Population number three was also the only population where the collection time in 1 year had an effect in the production of 7-methyljuglone (data not shown).

The best population in the 3 years survey comes from a lake shore bog, sphagnum substrate (Fig. 1, area number four). Concentrations of 7-methyljuglone in this population were always among the highest and the variation between years was quite low.

The results from these analyses indicate that there are differences in the production of 7-methyljuglone in *Drosera rotundifolia* L. in different regions in Finland. There are few published results of the concentration of 7-methyljuglone in *D. rotundifolia* of different origins (Bonnet et al., 1984; Caniato et al., 1989; Repčák et al., 2000). The concentration of 7-methyljuglone in most of the Finnish *D. rotundifolia* populations was higher when compared with one population from Italy (Caniato et al., 1989). The variation in secondary metabolite content of *D. rotundifolia* from various regions is in accordance with research made with other plant species. The content of secondary substances can vary between biotypes and populations from relatively close areas. Elevated temperature can enhance the production in experimental conditions and photoperiod also has an effect on secondary metabolism (Jensen et al., 1995; D'Antuono et al., 2000; Voirin et al., 1990).

There was no visible north–south trend in the concentration of 7-methyljuglone. In the far north areas with continuous light during the short growing season, the production was not significantly different when compared to more southern areas with longer growing season and shorter daylength. Weather conditions during the growth season varied a lot between years especially in the northernmost areas during the study period. Summer was cool and rainy in the first year in the most Northern parts of Finland and the last year of experiment was the opposite; extremely dry and much warmer (Fig. 2). The concentration of 7-methyljuglone was elevated in both of the most northern areas studied in the last year of experiment. To what extent the weather conditions affect the production of 7-methyljuglone remains open, but favourable conditions for photosynthesis seem to increase the allocation of energy for defence compounds, too. In cases where statistically significant correlations were found total rain amount per month in the growing season correlated negatively and mean temperature in July positively with the measured production of 7-methyljuglone (Pearson Correlations, Table 3). There were five populations with significant correlation between the weather conditions (mean temperature and total rain amount during the growth season months June, July and August) and the production of 7-methyljuglone (Table 3). These populations were from sand substrate (populations two and five) and the northernmost peat substrate population studied. Only two significant correlations were found from the other peat substrate populations (Table 3). In the sand substrate locations weather conditions seem to have more effects on the production of secondary metabolites than in the peat substrate locations.

From these results, the variation in the production of 7-methyljuglone among different populations of round-leaved sundew in Northern Finland is quite low. In each experimental year the overall variations between different populations in the production of 7-methyljuglone were statistically significant (ANOVA-tests), but in pairwise comparisons (Bonferroni-test) only few significant differences could be seen (Table 4).

The role of 7-methyljuglone for sundews is not quite clear. Plants produce carbon-based secondary defence substances in areas where there is a deficiency of nitrogen. Sundews inhabit nitrogen-poor locations and presumably they produce carbon-based naphthoquinones for defence. These compounds are toxic to certain types of bacteria and fungi and inhibit their growth. One suggested reason for the production of 7-methyljuglone is that it decreases competition of nitrogen from captured insects between plants and bacteria and fungi in the surface of these insects (Durand and Zenk, 1974).

According to present results of a population for cultivation should preferably be based on characteristics other than the production of naphthoquinones. The characteristics which need to be examined consist of production of biomass, wintering ability and seed production in various cultivation conditions.

The use of micropropagation enables the selection and multiplication of individuals, which produce higher than average amounts of 7-methyljuglone. Establishment of cultures from the cloned best individuals could lead to enhanced production of 7-methyljuglone. Further studies are being focused on these aspects.

3. Experimental

Round-leaved sundews (*Drosera rotundifolia* L.) were collected from 13 regions in Northern Finland (64–68°N) during the flowering season in July–August. The major part of the populations grow in different types of peat bogs, but also some sandpit habitats were included. Descriptions of the habitats and locations of different populations are presented in Table 5. Precipitation and temperature measurements (Finnish Meteorological Institute, 1995a, b, c, 1996a, b, c, 1997a, b, c) during growing seasons from four different latitudes near the study sites located meteorological observation stations are presented in Fig. 2.

The analysis of 7-methyljuglone was made from randomly chosen plants collected from the wild. The plants

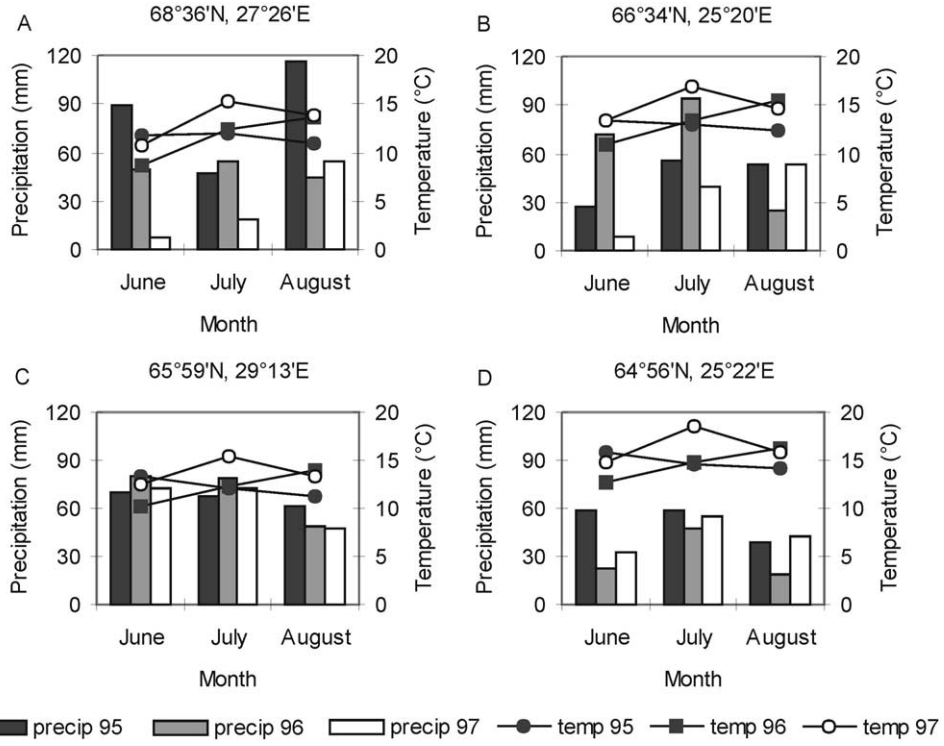

Fig. 2. Total precipitation values and mean temperatures from meteorological observation stations in different latitudes (A–D) in Finland during the growth season in the experiment years. ■ = total monthly precipitation in year 1995, ▒ = total monthly precipitation in year 1996, □ = total monthly precipitation in year 1997 ● = mean monthly temperature in year 1995, ■ = mean monthly temperature in year 1996 and ○ = mean monthly temperature in year 1997.

Table 3
The correlations between mean temperature, total amount of rain during the growth season months (June–August) and the production of 7-methyljuglone

Population	Production of 7-methyljuglone					
	Temperature			Rain		
	June	July	August	June	July	August
1	−0.004	0.489*	–	−0.180	0.023	–
2	−0.542*	0.668**	–	−0.697**	−0.512*	–
3	0.137	0.474	0.542	−0.212	−0.050	−0.726**
5	0.812**	0.174	−0.644	−0.725**	−0.725**	–
9	−0.763*	0.763*	0.763*	−0.763*	−0.763*	−0.763*

Results are shown only from populations where significant correlations (Pearson Correlation, two-tailed level of significance marked * = 0.05, and ** = 0.01) were found. Cases were correlation tests cannot be done are indicated as –.

were put into small plastic bags in the field and kept cold until sample preparation. The samples were prepared by weighing enough plants to make samples of approx. 1 g (4–58 plants per sample). Results of chemical analyses are averages of 2–12 replicates (n = in Fig. 1). During the first 2 years also non-flowering plants were taken for the analysis, because not enough flowering plants were available in some populations. Only flowering plants were analysed in the third experimental year.

Table 4
Results of the statistical analysis (ANOVA, Bonferroni-tests). Comparisons in 7-methyljuglone concentration in different populations in each year of experiment

Year		ANOVA		Bonferroni			
		F	Sig.	Population	Numbers	Mean difference	Sig.
1	n = 13	4.749	0.000	2	1	−0.578	0.046
				2	4	−0.735	0.001
				5	2	1.272	0.000
				5	12	1.164	0.009
				5	10	1.001	0.007
				5	6	1.023	0.021
				5	13	1.187	0.007
				5	8	0.955	0.014
2	n = 7	12.329	0.000	4	2	0.539	0.022
				4	7	0.739	0.000
				4	5	0.780	0.000
				6	7	0.582	0.000
				6	5	0.623	0.001
3	n = 8	3.641	0.007	2	3	−0.631	0.030
				2	9	−0.596	0.050

Only significant differences are shown as Bonferroni-test results.

The dead leaves and roots from previous years growth were cut off before measuring the fresh weight and only the current year growth, without roots, was used for the analysis. The small size of an sundew (in population 10

Table 5
Locations and description of the habitats of different populations. Growing season indicates the average number of days per year in which the mean temperature reaches + 5°C

Population number	Location	Growing season (days)	Daylength (h)			Description
			June	July	August	
1	65°08′ N, 25°54′ E	145	22	20	17	*Sphagnum fuscum* dominated bog in border of eutrophic lake
2	65°14′ N, 25°53′ E	145	22	20	17	Abandoned sandpit in border of a oligotrophic pond
3	65°15′ N, 26°29′ E	140	22	20	17	*Sphagnum fuscum* substrate hummocks in *Carex* bog
4	65°03′ N, 27°47′ E	140	22	20	17	Lake shore bog, *Sphagnum fuscum* hummocks in or near the water level
5	66°29′ N, 25°20′ E	130	24	21	17	Abandoned sandpit surrounded by *Sphagnum fuscum* bog
6	66°30′ N, 25°22′ E	130	24	21	17	Open *Sphagnum fuscum* bog
7	64°41′ N, 25d23′ E	150	22	20	17	*Pinus* bog, hummocks with dense coverage by *Empetrum nigrum*
8	67°33′ N, 26°37′ E	125	24	24	18	Open *Sphagnum fuscum* bog with water pits
9	68°30′ N, 27°35′ E	120	24	24	18	*Sphagnum fuscum* dominated hummocks in border of a small pond
10	66°20′ N, 29°18′ E	125	23	20	17	Open *Sphagnum fuscum* bog with water pits
11	65°23′ N, 25°23′ E	145	22	20	17	Open *Pinus* bog with *Sphagnum fuscum* hummocks
12	65°35′ N, 28°28′ E	140	23	20	17	Open *Sphagnum fuscum* bog between *Pinus* bog and a oligotrophic lake
13	64°24′ N, 24°50′ E	150	22	20	17	Open *Sphagnum fuscum* bog

mean weight of a plant was 35 mg) did not allow measurements of 7-methyljuglone content of different plant individuals in some populations with extraction method used in this study. Dry weights from each population were obtained from additional samples dried in + 80 °C until the weight was stable.

Extraction of 7-methyljuglone was done according to Bonnet et al. (1984) with minor modifications. Briefly, fresh plant material (approx. 1 g) was homogenized with a mortar and a pestle in liquid nitrogen. One milligram juglone (Sigma) in 1 ml toluene was added as internal standard. The sample was extracted three times with 10 ml toluene. The toluene fractions were combined, filtered through Whatman no.1 filter paper and the solvent was evaporated using vacuum evaporator. The orange-brown residue was dissolved in 1 ml acetonitrile.

Gas chromatography was performed with Carlo Erba HRGC eqiuped with flame ionization detector (FID; H_2: 0.45 kg cm^{-1}, air: 0.80 kg cm^{-1}). Fused-silica capillary column was DP-624 (25 m, 0.323 mm i.d., film thickness 1.80 μm) and carrier gas was helium (0.66 kg cm^{-1}). Oven temperature was isothermic 250 °C, injector temperature was 240 °C and detector temperature was 260 °C. One microlitre of each sundew sample (in acetonitrile) was split-injected (split flow 7 ml min^{-1}, septum purge 1.5 ml min^{-1}) once. The CG/FID data were collected and the peaks integrated using Kontron Instruments PC Integration Pack version 2.50 (Softron GmbH). The retention times were 4.1 and 5.2 min for juglone and 7-methyljuglone, respectively.

The naphthoquinone of the *D. rotundifolia* was identified to be 7-methyljuglone with GC/MS experiments using Saturn 2000 (Varian) ITD mass spectrometer. The mass spectrum obtained for 7-methyljuglone was similar to those measured by Bonnet et al. (1984) and Budzianowski (1995). No other naphthoquinones were found. Plumbagin (Sigma) standard was also injected to ensure that the found naphthoquinone was not plumbagin. As a result, the mass spectra were different and also the retention times were different between plumbagin (5.0 min) and 7-methyljuglone (5.2 min).

The quantitation of 7-methyljuglone was performed according to Bonnet et al. (1984) by direct comparison of GC/FID peak areas of internal standard juglone and 7-methyljuglone. The relative response factors of juglone and 7-methyljuglone were supposed to be equal when using FID for detection. No calibration curves were done for juglone, but the content of 7-methyljuglone was calculated as equivalents of internal standard. The calculation was done separately for every sample. External calibration using 7-methyljuglone was not applicable as it is not available commercially. Plumbagin was not used as internal standard, because its retention time was too close to that of 7-methyljuglone.

Analysis of variance [ANOVA, SPSS (*r*) for Windows], two independent samples *t*-test, Bonferroni test, and Pearson Correlation test were used in the statistical analysis of the data. Levene test was used to test the homogenity of variances, Kolmogorow–Smirnov test and Shapiro–Wilk test were used to test the normality of the data.

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