

Review article

## **Acclimatization studies with cold tolerant medicinal plants in Finland**

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### Abstract

In order to find new alternative crops suitable for the Nordic climate, a series of acclimatization agronomic experiments were carried out with cold tolerant medicinal plants at Mikkeli, Finland ( 61°44 N , 27°18 E) during 1985-1999. Medicinal plants with adaptogen effects ( *Leuzea carthamoides* L., and *Rhodiola rosea* L.), essential oil containing species with anthelmintic and insect repellent effects ( *Artemisia abrotanum* L. and *Myrica gale* L.), and plants from the high mountains of the Alps for medicinal and aromatic uses ( *Arnica montana* L. and *Gentiana lutea* L.) were studied.

Growth rhythm, overwintering, biomass potential, and the accumulation of secondary metabolites were determined and basic agronomic techniques developed.

Keywords: acclimatization, cold tolerant medicinal plants, cultivation methods, *Arnica montana* L., *Artemisia abrotanum* L., *Gentiana lutea* L., *Leuzea carthamoides* DC., *Myrica gale* L., *Rhodiola rosea* L.

### Introduction

Finland is one of the northernmost agricultural countries in the world, and its geographical situation limits the possibilities of plant production. In order to find alternative special crops suitable for Nordic climatic conditions, a large number of experiments was carried out during the last decade in our institute ( Kaukovirta- Galambosi, 1996 ). Traditional herbs of Mediterranean origin seemed to

give low yields under Finnish conditions, therefore the focus of herb research was directed to the introduction of cold tolerant medicinal plants and herbs into Finland.

Plants, which were expected to tolerate the cool climatic conditions, were chosen from biogeographically homologous areas to Southern Finland, where the climatic conditions are similar to Finland, such as the high mountain areas of the Alps and the Carpatians in Europe, and South Siberia. Additionally, some native species of the Finnish flora were chosen. According to the literature, all chosen plants have some special biological activities.

The aim of this acclimatization study was to obtain information on their suitability to Finnish climatic conditions, to have data on their growth and biomass production, as well as on the quantity and quality of the secondary metabolites presented in the literature .

## Material and methods

### Plants species:

The medicinal and aromatic plant species cultivated in this study are presented in Table 1. Only two of them are endemic in the Finnish flora ( *Myrica gale* L. and *Rhodiola rosea* L ).

**Table 1. Cold tolerant new medicinal plants studied in the acclimatization experiments at Mikkeli, Finland during 1985-1999.**

Plant			Experimental year
<i>Gentiana lutea</i> L.	( <i>Gentianaceae</i> )	non-endemic	1985-1999
<i>Arnica montana</i> L.	( <i>Asteraceae</i> )	non-endemic	1990-1999
<i>Artemisia abrotanum</i> L.	( <i>Asteraceae</i> )	non-endemic	1996-1999
<i>Leuzea carthamoides</i> DC.	( <i>Asteraceae</i> )	non-endemic	1990-1999
<i>Myrica gale</i> L.	( <i>Myricaceae</i> )	endemic	1995-1999
<i>Rhodiola rosea</i> L.	( <i>Crassulacae</i> )	endemic	1990-1999

### Cultivation conditions:

The plants were cultivated in the experimental fields of Ecological Production, Karila, Research Management Research, Agricultural Research Centre of Finland, at Mikkeli (grid reference 61° 44 N, 27° 18 E) during 1985-1999. The soil was a stony till, pH 6.2. The experimental plots of *Artemisia* and *Leuzea* species were fertilized with a mixed fertilizer ( NPK =35-120-70 kg/ha) before planting with further nitrogen ( 15 kg/ha) three weeks after planting. Weeding was carried

out mechanically. *Arnica*, *Gentiana*, *Rhodiola* and *Myrica* species were transplanted into plots covered with plastic mulch and before spraying of the mulch, the plots were fertilized by 20 t/ha compost. During the experimental period, biological and agrotechnical experiments were carried out. The size of the experimental plots varied from 2 m<sup>2</sup> to 40 m<sup>2</sup>, in three or four repetitions.

#### Measurements:

Plant growth, flowering and overwintering were observed, plant height, weight, fresh and dry yields, dry matter content and stem/leaf ratios were measured. The contents of the flavonoids of *Rhodiola rosea* and ecdisteroids of the *Leuzea carthamoides*, were determined in the Department of Pharmacognosy, the University of Szeged, Hungary. The essential oil content and composition of *Artemisia abrotanum* and *Myrica gale*, as well as the antioxidant properties of *Arnica montana* were determined in the Scottish Agricultural College, Ayr. The analytical methods are presented in separate publications.

#### Results and discussion:

##### *Arnica montana* L.

*Arnica montana* is a perennial medicinal plant originated from the high mountains of the Alps and the Carpatians. Its flower contains volatile oils, terpenoides, sesquiterpenes lactones, flavonoides, bitter principles with antiseptic, antifungal, antibiotic and antioxidant effects.(Carol et al. 1996) The estimated consumption of the dry flower yield is about 20-30 t/year. Due to its intensive collection from the nature, it is now a rare and endangered species in the wild (Lange 1998).

Due to its cold tolerant characteristics, it grows well in Finland.(Galambosi et al,1998). It is propagated by seeds and the seedlings may be transplanted into black plastic mulch, reducing the weed control cost during its 4-5 years of growth. The phenologies and the yield components are presented in Table 2. *Arnica montana* generally flowers at Mikkeli during July and produces an acceptable flower yield in an organic cultivation system. The expected yield of dried flowers ranges between 2-11 kg/100 m<sup>2</sup>. According to the analytical results, the flowers collected in different years and different times showed moderate and marked antioxidant activities (Table 3).

**Table 2. Phenologies and yield components of stands of *Arnica montana* in four growing seasons cultivated in Finland. (Galambosi et al. 1998).**

	1990	1991	1992	1993
Start of flowering	21.6.	26.6.	13.6.	13.6.
End of flowering	11.7.	23.7.	31.7.	23.7.
Plant height cm	40	37.5	45	53.3
Plant rosettes diameter cm	13	15	22	37
No. stems per plant	1 - 2	5 - 7	5 - 35	5 - 35
No. flowers per plant	5	28	77	98
Total fresh flower weight g/plant		33.1	80.1	113.7
Total dry flower weight g/plant		5.8	14.6	19.2
Fresh flower yield g/m <sup>2</sup>		198.3	480.4	682.2
Dry flower yield g/m <sup>2</sup>		34.9	87.4	115.2

**Table 3. Antioxidant properties of dichloromethane extracts of flowers.**

(Date of Harvest/Zone of Antioxidant Activity\* /mm/ and Colour Retention index\*\*) (Galambosi et al. 1998)

1991			1992			1993		
4 July	13.5	∞∞	22 June	11.4	∞	25 June	12.0	∞
6 July	11.4	∞	24 June	9.1	∞	27 June	11.0	∞∞
8 July	15.0	∞∞∞	26 June	13.3	∞∞	29 June	9.7	∞
			29 June	15.3	∞	2 July	12.5	∞∞
			9 July	8.3	∞			
			11 July	13.1	∞∞			

*Gentiana lutea* L.

*Gentiana lutea* is a perennial medicinal plant originated from the same ecological origin as arnica (the Alps, the Carpatians, mountains of Massive Central in France). Due to its aromatic and bitter constituents, like gentiopicrosides, its root is a significant raw material in the pharmaceutical and

liqueur industry (Carol et al, 1996). The major part of the annual industrial consumption (more than 2000 tons per year) is collected from the wild, but presently its cultivation has been started in Europe (Lange, 1998).

On the basis of our cultivation experiments, it seems that yellow gentian can be successfully grown in Finland. The mother plants can produce fertile seeds for own use, the plantations can be founded by transplanting one-year-old seedlings. The plants produce a root yield suitable for harvest after 4 or 5 growing years (Figure 1). Black plastic mulch has proved to be a very effective method of weed-control for the slowly growing gentian. Using a plant density of 9 pots/m<sup>2</sup>, the average fresh root yields after the 4th and 5th growing years were 5.7 and 8 kg/m<sup>2</sup>, respectively (Figure 2)(Galambosi,1996).

Figure 1. Development of root weight of *Gentiana lutea* during 1984-1989 at Puumala,Finland. (Galambosi, 1996)

Year	3	4	5	6	3	4	5
Dry	1.16	1.13	1.78	1.6	2	1.9	2.13
Fresh	4.67	4.52	7.14	6.4	8.03	7.61	8.54

Figure 2. Root yield of *Gentiana lutea* after the 4<sup>th</sup> and 5<sup>th</sup> growing years.Mikkeli,1994-1995.

	1	2	3	4	5	6
dry			94	159	267	415
fresh			363	619	1338	1848

### *Artemisia abrotanum* L

*Artemisia abrotanum* is a perennial, erect-growing aromatic scrub, native to southern Europe. The content of essential oil in the dried leaves is 0.6-0.9 % with 20-66 % of 1,8-cineol as a main compound (Vostrowsky et al. 1984). Due to its aromatic properties, it was used as an anthelmintic, digestive, cholagogue, emmenagogue. As a scrub, *Artemisia abrotanum* was always used as a decorative perennial. Its anthelmintic effect was studied in a German study, in which good results were obtained with its homeopathic remedy for dogs and cats infected with gastrointestinal nematodes (Krause, 1993).

Contrary to its Mediterranean origin, it tolerated quite well the winters at Mikkeli and overwintered 2-3 consequent winters. It is propagated by stem cuttings of 10 cm length, cut in spring from the overwintered mother plants and they are easily rooted in moist sand. The seedlings can be planted into field using 60 x 40 cm plant density or in plastic mulch 5 plants/m<sup>2</sup>. Its cultivation for dry drug or essential oil production could be mechanized. The essential oil of the dried leaf yield was 1.2 %,

reaching its maximum at the start of flowering at the beginning of August. The main compound of the oil was 1,8 cineol (57.4 %). The total fresh biomass of the first and the second year old plantations was 1.1 and 1.8 kg/m<sup>2</sup>, respectively.

### *Leuzea carthamoides* DC.

*Leuzea carthamoides* DC. is a perennial medicinal plant of Siberian origin. The plant has a woody rhizome with wiry roots of a length of 20- 40 cm. The leaves are oval or elliptical, 10.30 cm long. The flower stems, 1-3 per plant, appear in the second year, their height ranges between 80-170 cm. The 1000 seed weight is 11-19 grams.

Its roots, which have a novel type of pharmacological action classified as adaptogenic, are commonly used for medicinal purposes in Russia. Ecdysteroids, flavonoids, polyacetylenes and triterpenes have been isolated from its roots, leaves and seeds. (Girault et al. 1988). Of the isolated compounds, 20-hydroxyecdysone was investigated for biological activity. (Syrov -Kurmukov, 1976.) The alcoholic extracts of the roots considerably increase the capacity of tired skeletal muscles, enhance the resynthesis of glycogen and high energy phosphorus compounds.

Preparations produced from *Leuzea* in the former USSR are "Ekdisen", in Czech Republic: "Maralan", "Leuzea" in Slovak Republic, "Robofit" in Hungary. Preparations used by athletes ("Triboxin") are claimed to increase endurance, reflexes and concentration, and they were regenerated earlier. The above ground parts of the plant contain high value fodder material (Földesi et al, 1982)..

Based on experiments from 6 years, *Leuzea* has been successfully introduced into Finland as a novel crop with special biological properties. It has shown a good adaptability to the Finnish climatic and soil conditions. Its overwintering was safe and its biomass potential was the same as reported earlier. The experimental dry root yields in the second and third year were 0.2-0.5 kg/m<sup>2</sup> and 0.6-1.0 kg/m<sup>2</sup>, respectively. ( Figure 3). In fertilization experiments, where the following fertilizer levels were used L<sub>1</sub>:NPK=55-0-0, L<sub>2</sub>: NPK=145-50-90 , L<sub>3</sub>: NPK=210-80-145 kg/ha, respectively, the fresh and dry herb yields of the experimental plots during the second and third year ranged between 25- and 35 to /ha and 4- and 5.5 to /ha, respectively ( Figure 4). Regarding the chemical composition, the 2-and 3-year old plants grown in Finland contained the same quantities of 20-

hydroxyecdysone as reported earlier in the Russian and Hungarian studies. It produces seeds of good biological value (Galambosi, et al, 1997).

Figure 3. Dry root yield of transplanted *Leuzea carthamoides* in potato ridges. Mikkeli,1991-1993. (Galambosi et al. 1997)

Year	1991	1992	1992	1992	1992	1993	1993	1993	1993	1993
Dry root yield	24.9	28.5	26.9	17.8	9.9	9.8	16.8	24.8	2.9	14.9
Kg/m <sup>2</sup>	0.14	0.25	0.27	0.49	0.42	0.75	0.62	0.65	0.90	1.02

Figure 4. Fresh and dry herb yield of *Leuzea carthamoides* at different fertilization levels. Mikkeli,1993-1995.(Galambosi et al. 1997)

	YEAR 1			YEAR 2			YEAR 3		
	L <sub>1</sub>	L <sub>2</sub>	L <sub>3</sub>	L <sub>1</sub>	L <sub>2</sub>	L <sub>3</sub>	L <sub>1</sub>	L <sub>2</sub>	L <sub>3</sub>
dry	1.37	1.41	1.37	2.68	4.26	5.53	2.32	2.50	4.80
fresh	8.9	10.3	9.8	13.5	24.5	33.0	11.8	13.5	23.5

### *Myrica gale L.*

*Myrica gale L.* is a common deciduous shrub of wet, acid soils in the Northern hemisphere (Europe, Siberia, Canada, and Northern USA). Its leaves and catkins contain a pleasant smelling essential oil in 0.05-0.97 %. The composition of the essential oil is presented in Table 4.

Dried leaves and fruits were used in the folklore for many purposes, e.g. as spices, as a flavoring for beer, to perfume linen, as a source of wax for candles or as an insect and flea repellent. ( Svoboda, et al, 1998). Recently there has been an increased interest from pharmaceutical companies in the volatile oil of *Myrica* for its use as a repellent against midges ( Michael et al. 1996). There are wide natural populations in the shores of inland lakes in Finland ( Hämet-Ahti et al. 1992)

Due to interest in *Myrica* as a raw material for industrial purposes, biochemical and agronomic experiments have been started in Scotland and in Finland for the determination of its possible biomass production and essential oil yield and quality ( Svoboda, et al, 1998).

According to our measurements, the total dry biomass production of the natural populations was in Scotland and in Finland 420 and 275 g/m<sup>2</sup>, respectively. The essential oil containing dry leaf yield was 250 and 180 g/m<sup>2</sup>, respectively. There was no variation in the essential oil content of the dried leaves which in both sites ranged between 0.15-0.22 %. The oil content of the flowers was higher ( 1.0-1.4 %) but their proportion in the total yields were very low.

Since the collection of the natural *Myrica* populations in the lake shores and swamp areas is expensive, agronomic studies have been started at Mikkeli to elaborate its growing methods in normal field conditions. According to our preliminary experiences obtained during 1997-1999, *Myrica gale* grows sufficiently in mineral soils. The studies focused on its mass propagation methods, on the regeneration of plants after mechanical harvest and on the possible increase of the low biomass production by different fertilization practices.

**Table 4. Chemical composition of the volatile oil of *Myrica gale* L. from various geographical sources. (Svoboda et al.1998).**

Constituent	%
<i>α</i> -pinene	3.0 - 41.4
<i>β</i> -pinene	0.1 - 1.8
camphene	0.1 - 0.7
myrcene	1.3 - 29.1
<i>α</i> -terpinene	0.1 - 5.6
<i>γ</i> -terpinene	0.0 - 1.8
limonene	1.5 - 14.6
1,8-cineole	0.1 - 20.0
<i>β</i> -phellandrene	0.0 - 3.2
<i>cis</i> -ocimene	0.0 - 3.6
<i>trans</i> -ocimene	0.0 - 4.4
<i>p</i> -cymene	0.0 - 6.0
copaene	0.0 - 2.8
linalol	0.0 - 1.3
terpinen-4-ol	0.1 - 2.2
caryophyllene	0.0 - 5.5
selina-4,11-diene	0.0 - 6.0
<i>α</i> -terpinol	0.1 - 4.0
<i>α</i> -humulene	0.0 - 3.4
<i>β</i> -bisabolene	0.0 - 3.0
<i>β</i> -cadinene	0.0 - 12.5
calamene	0.0 - 2.4
<i>trans</i> -nerolidol	0.0 - 5.9
caryophyllene oxide	0.0 - 1.8
eudesmol	0.0 - 3.5
<i>α</i> -bisabolol	0.0 - 5.0
selin-11-en-4-ol	0.0 - 14.6
<i>β</i> -elemene	0.0 - 1.0

nerol	0.0 - 7.7
nerolidol	0.0 - 4.0
$\beta$ -elemenene	0.0 - 14.3
isobarbatene ketone	0.0 - 4.4
germacrene	0.0 - 11.6

*Rhodiola rosea* L.

Roseroot, *Rhodiola rosea* L. (syn: *Sedum rosea*) is a dioecious, herbaceous perennial plant, originated in the mountain regions of South West China and native around whole of the northern hemisphere north of the 50<sup>th</sup> latitude. It grows as a natural plant in the Alps and the Carpatian mountains and in the Northern parts of Europe. It is a decorative, but slowly growing species, it is usually grown as a garden perennial. *Rhodiola rosea* L. is described as an adaptogen medicinal plant together with *Schiandra*, *Panax*, *Leuzea*, *Aralia*, and *Acanthopanax* ( Brekhman-Dardymov 1969, Ahumada et al, 1991).

Its roots and rhizomes contain different compounds, specific for the species: salidroside, cinnamyl alcohol glycosides (rosine, rosavine, rosarine), flavonoids,( rhodionine, rhodiosine, rhodioline), terpenoids, etc. Salidroside glycosides are considered to have stimulating and energy-giving effect on memory, stamina, and the ability to cope.

According to our first cultivation experiences, roseroot can be successfully grown for its root yield in South Finland using organic growing methods.( Galambosi et al, 1999) For seedling production, seeds have to be sown in autumn, for natural winter stratification. The seedlings should be kept in pots for one year before transplantation, since the growth of the plants during the first 2-3 years is quite slow. The first root yield was harvested after four years from sowing and the root weight and root yield strongly depends on the plant age ( see table 5 ).

**Table 5. Root yield of *Rhodiola rosea* L. plants of different origin. 1998, Mikkeli, Finland.**

Code/origin	Plant age year	Fresh weight g/pot	Dry matter %
A <sub>1</sub> Finnish, Hirvas	4	410	26
B <sub>1</sub> Norwegian	4	487	23
B <sub>4</sub> Swedish, Impecta	4	497	23
C <sub>2</sub> Germany, Kiel	4	469	26
C <sub>8</sub> Finnish, Raahe	4	409	26
<b>MEAN</b>		<b>454</b>	<b>25</b>

<b>B<sub>3</sub> Finnish, Särkä</b>	<b>5</b>	<b>1080</b>	<b>25</b>
<b>C<sub>3</sub> Austria</b>	<b>5</b>	<b>1368</b>	<b>24</b>
<b>MEAN</b>		<b>1224</b>	<b>25</b>

The fresh root yield of the four year old plants ranged between 1.8-2.8 kg/m<sup>2</sup>, that of the six-year-old plants ranged between 7.6-10 kg/m<sup>2</sup>. The sliced thick rhizomes dried very slowly, during 5-7 days. The dry root yield of the four-year-old plants was 0.4-0.6 kg/m<sup>2</sup> and of the six-year-old plants 1.6 kg/m<sup>2</sup>. There was great morphological variability among the roseroot plants of different origins, which requires additional agronomic and chemical investigations.

Conclusion:

Introduction of new plant species with different economical value into the Finnish flora and agronomic practices during the history have always had great importance. Using a system of the bioclimatic vegetation zones, developed by Finnish botanists (Hämet- Ahti & Ahti, 1969), successful acclimatization experiments were carried out with those medicinal plants, which originate from climatically similar zones to Finland. Nearly all plants grown in this study originated from climatic zones in which the dominating climatic characteristic are the short summer, long winter periods with low annual temperature and highly alternating daily temperatures. There were no winter damages observed in the plantations, except in *Artemisia abrotanum*, a species originated from the Mediterranean area. Species originally belonging to the Finnish flora show a good adaptability to the more intensive growing conditions in fields and can grow without any damage even when fertilized. (Galambosi 1993). The quantity and the quality of the biomass plants obtained in cultivation experiments carried out in Finland show similar results as are reported in the literature.

The introduction and acclimatization of any new plant species into a new environment is a long-term research. On the basis of our 5-6 year observations and experimental results, we created the first version of growing system of the studied plants and we checked these systems in semi large-scale cultivation plots. During 1997 experimental cultivation plots were founded in a size of 400 m<sup>2</sup> for *Arnica montana*, *Gentiana lutea*, *Rhodiola rosea* and in a size of 2000 m<sup>2</sup> for *Leuzea carthamoides*. The cultivation system of *Myrica gale* needs additional research.

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