

# Feasibility of Cultivation *Calendula* as a Dual Purpose Industrial Oilseed and Medicinal Crop

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**Keywords:** *Calendula officinalis*, calendic acid, field trials, oil content, flavonoids, flower removal

## Abstract

*Calendula* (*Calendula officinalis* L.)(Asteraceae) flowers have been used as anti-inflammatory and wound healing agents for hundreds of years. More recently, the calendula seed was found to contain up to 60 % calendic acid (C18:3 $\Delta^{8,10,12c}$ ), which because of its high rate of oxidation has numerous industrial applications. Two cultivars of calendula, 'Resina' and 'Erfurter Orangefarbigen' (EO) were evaluated for the feasibility of combined seed and flower production in western Canada. Results from a two-year study indicated that both cultivars can produce a reasonable seed crop (400 – 950 kg/ha) on the prairies, if planted by the middle of May. 'Resina' was found to be higher seed yielding than 'EO' during both years of the study. Seed of both cultivars contained on average 15 % (w/w) of oil with calendic acid accounting for 50 – 55 % of the oil. These levels are similar to those reported in Europe. Seed yield was not affected by flower removal after the middle of August. Flavonoid content in flowers determined by HPLC and expressed as isorhamnetin glycosides was in the range of 0.62 % to 1.73 %. It was substantially higher in 'EO' than 'Resina'. Flavonoid content in both cultivars decreased progressively from mid-August to mid-September. *Calendula* has potential for cultivation as a dual purpose crop in western Canada.

## INTRODUCTION

*Calendula* is a flowering annual with an indeterminate flowering habit that is grown as an ornamental and medicinal plant throughout most regions of the world. The flowers have been used as a source of medicinal ingredients and dyes for centuries. While active constituents in flowers are not known, compounds of medical interest believed to contribute to the anti-inflammatory and immuno-stimulatory properties include triterpenes, flavonoids, carotenoids, polysaccharides, and sterols (Janiszowska, 1987; Della Loggia, 1994). Flavonoids are believed to account for much of flowers anti-inflammatory activity and are considered to be main biochemical markers for calendula destined for herbal drug market.

Most calendula research has focused on extraction and/or pharmacological properties of bioactive compounds from flowers. More recently, calendula has been reported to accumulate an unusual conjugated C<sub>18</sub> fatty acid named calendic acid (octadeca-8:10:12-trienoic acid) in its seed (Beerentrup and Röbbelen, 1987). Due to the rapid oxidation, calendic acid has numerous potential industrial applications such as drying agent in paints, varnishes and plastics. It has been estimated that calendic acid could replace a number of hazardous volatile organic compounds (VOC) with potential market of 3-5 million kilograms annually in western Europe alone (Capelle, 1996).

It has been reported that calendula yields between 700 and 2400 kg of seed per hectare in Europe (Breemhaar and Bouman, 1995; Cromack and Smith, 1998; Rexen and Blicher-Mathiesen, 1998). The seed contain 15-20 % oil (w/w) of which calendic acid accounts for 50 – 60 % (Breemhaar and Bouman, 1995). The purpose of this research was twofold. Firstly, to determine whether calendula can be grown under dryland farming conditions in western Canada, specifically Saskatchewan (SK) and Alberta (AB), and secondly, whether it is feasible to grow calendula for dual purpose: seed for industrial

applications and flowers for medicinal applications.

## **MATERIALS AND METHODS**

### **Location**

The studies were conducted at Saskatoon, SK (52° 9' N, 106° W, 515 m elevation), and Brooks, AB (50° 33' N, 111° 51' W, 758 m elevation), Canada, in 1999 and 2000. Saskatoon plots were in orthic dark brown chernozem and Brooks plots in brown chernozem soil. The soil fertility level at both test sites in both test years was considered adequate ( $> 80$  kg/ha  $\text{NO}_3\text{-N}$ ,  $> 150$  kg/ha P and  $> 1000$  kg/ha K), and no additional fertiliser was provided.

### **Field Studies**

**Cultivar x Seeding Rate Study:** It was conducted using two cultivars, 'Resina' (yellow flowered, early maturing and large seeded; Johnny's Selected Seed, Albion, ME) and 'Erfurter Orangefarbigen' ('EO') (orange flowered, late maturing and small seeded; Richters, Goodwood, ON). The crop was seeded at 6 kg/ha and 12 kg/ha on May 3, May 18 and May 31 in Saskatoon in 1999 and 2000 and in Brooks in 2000, and on May 18 and May 31 in Brooks in 1999. Treatments were arranged in split-plot design with four replications, the seeding rate treatment as main-plot and cultivar as sub-plot. Each treatment consisted of six 3.6-m rows spaced at 18 cm in Brooks and four 4-m rows spaced at 30 cm in Saskatoon. In Saskatoon, calendula was grown with no irrigation, and in Brooks under rain-fed conditions in 1999 and with supplementary irrigation in 2000. The plant population density was determined at flowering by counting the number of plants per linear meter from two middle rows of each plot. Seed was harvested 115-120 days after seeding. At harvest, plant height and plot seed weight (fresh and dry weight) were determined.

**Cultivar x Flower Harvest Date Study:** It was conducted using 'EO' (Brooks and Saskatoon) and 'Resina' (Saskatoon) at a seeding rate of 6 kg/ha. Flowers were harvested at full bloom over a four week period starting August 16 and dried at 40 °C until moisture content was below 10 %. Treatments were arranged in a randomised complete block design with 4 replications. The plot size and row spacing were similar to those for study 1 at both test sites. The plant desiccant diquat (Reglone) was foliar applied at recommended rate about one week before crop harvest. Data collection was similar to that for study 1. Data were subject to ANOVA and mean comparisons using LSD test.

### **Oil Extraction and Fatty Acid Analysis**

Oil content was determined in 10 grams of ground seed (particle size 1000  $\mu\text{m}$ ) by Soxhlet extraction with hexane. Fatty acid composition of the extracted oil was determined by GC. Also, single seeds were randomly selected from the various plots, baked @ 100 °C for 1 h in a methanol solution containing 2 % sulfuric acid, and then extracted with hexane. The hexane extract was injected into a J&W DB-23 column installed in an HP 5890 Series II GC using an isothermic temperature of 220 °C and helium as a carrier gas at a flow rate of 1 ml/min. Fatty acid identification was based on retention times of known standards.

### **Flavonoid Extraction and Analyses**

Dried calendula flowers from Saskatoon and Brooks were ground in a coffee grinder and passed through a 24-mesh sieve prior to chemical analyses. Ground flowers (500 mg) were extracted with 50 mL of 50 % (v/v) methanol at 40 °C over 2 h in a water bath shaker and an aliquot of each extract was centrifuged for 15 min @12,000 RPM. The total flavonoid content was determined in the supernatant according to a spectrophotometric method of Hedin et al. (1992) using isorhamnetin as a standard. Hedin's method is based on chemical derivatization of flavonoids with diphenyl borinic acid ethanolamine ester and absorbance measurement of the colored product at 440 nm.

An aliquot of each extract was also subjected to flavonoid profiling by HPLC using Waters HPLC system equipped with an autoinjector, diode array detector and Waters Novapak<sup>TM</sup> RP<sub>18</sub> column (5 $\mu$ m, 150x3.9 mm). Method of Pietta et al. (1992) was applied with some modification. Flavonoids were separated within 10 min using isocratic elution with a mobile phase consisting of 14 % (v/v) isopropanol (solvent A) and 86 % of 5 % tetrahydrofuran in water (solvent B) at a flow rate of 1.2 ml/min; detection was done at 356 nm. Identification of flavonoids was based on retention times of known reference compounds: isorhamnetin (I), isorhamnetin-3-rutinoside (I-3-R), isorhamnetin-3-glucoside (I-3-G), quercetin and kaempferol. Isorhamnetin-3-O-2-rhamnosyl-rutinoside (I-3-R-R) was identified in calendula flower extract in our earlier studies by LC-MS-MS. The content of I-3-R-R was estimated using the calibration curve for I-3-R corrected for the difference in molecular weight. Analysis of flavonoid aglycones was conducted by HPLC on plant extracts (3-4 mg) upon acid hydrolysis with 25 % HCl for 30 min at 85 °C as described by Akhrov and Barl (2002). Data was collected and processed digitally with Millennium<sup>32</sup> chromatography software.

## RESULTS AND DISCUSSION

### Yield Trials: Seeding Date, Seeding Depth, and Seeding Density

Seed yield of calendula seeded on May 3, May 18, and May 31 at both locations (except in Brooks in 1999 when May 3 seeding was omitted) was consistent for both cultivars and all seeding dates. The 'Resina' out yielded 'EO' at both locations in both years with average seed yield of 765 kg/ha (Table 1). Site effect was insignificant. Seeding density had no effect on yield at two seeding rates tested (Table 1). At the lower density of 6 kg/ha the plants compensated by producing a greater number of lateral branches such that total flower number was unchanged in comparison to seeding density of 12 kg/ha. Also, no differences were observed in any of the other measured parameters at the two seeding densities tested. Seeding depth studies were conducted only at Saskatoon and no differences in seed yield, plant density, plant height or days to flowering were observed when crop was seeded at 1.25 or 2.5 cm (data not presented).

To the best of our knowledge, this is the first study on cultivation of calendula in North America that reported seed yield data. Berti and Schneiter (1993) indicated the potential for cultivation of this crop in North Dakota, but provided no information on yield. Callan et al. (1999) found that calendula could be successfully grown in Montana for flower production. Calendic acid content in the seed oil (49-54 %) and total flower dry weight (127 kg/ha) were reported, but not the seed yield.

In this study the yields of calendula were either below or at the lower end of the range reported in European trials (700 kg ha<sup>-1</sup> to 2800 kg ha<sup>-1</sup>) (Beerentrup and Röbbelen, 1987; Breemhaar and Bouman, 1995; Cromack and Smith, 1998; Rexen and Blicher-Mathiesen, 1998). Careful comparative analysis of the data of European and our studies indicated that the yield differences were not as great as they appear. The highest reported European yields resulted from multiple hand harvest of seeds as the flowers matured over a 7-week period (Beerentrup and Röbbelen, 1987). Manual harvesting is, however, not practical in a large scale operation plus it minimizes losses due to seed shattering. We conducted preliminary assessment of combine-induced shattering losses by placing a plastic sheet on the ground prior to combining and weighing collected seed from the plastic sheet upon harvest. Seed losses were found to exceed 30 %. Additional shattering losses occurred during one trial in Brooks when plants were sprayed with Reglone, but combining had to be delayed for 3 weeks due to wet weather following desiccation. These plots yielded less than 35 % of the neighbouring plots that were harvested prior to the onset of rain. Use of weed control is another possible source of yield differences. In the European studies, a pre-emergent herbicide treatment was applied, whereas in our studies seeding was conducted into untreated plots.

The final yield-reducing factor was the infestation of aster yellows, a phytoplasma disease transmitted by leafhoppers. At both Saskatoon and Brooks site, most plots showed

signs of infection with the 'Resina' displaying a higher percentage of malformed floral buds. Callan et al. (1999) also indicated that aster yellows was a serious problem in their study in Montana. Chemical control of leafhoppers is a possible solution, but it would be of far greater benefit to find resistance to this disease within the calendula germplasm.

### **Flower Removal Trial**

Calendula flowers have been utilized traditionally in herbal and/or dermatological preparations to treat inflammations of the skin and mucosa, and as an aid to wound healing (ESCOP Monographs, Fascicule 1, 1996). It was of interest to determine whether flower production could be combined with the seed production of calendula, set as the primary goal of this project. Fully opened flowers were removed from plants either mechanically or manually at weekly intervals beginning in mid August, since these flowers would not have sufficient time to form mature seed by the time of seed harvest in mid September.

Flower removal even at the earliest removal date of August 16 resulted in no seed yield reduction in comparison to control for both cultivars at both locations (Table 2). An average seed yield of 'Resina' and 'EO' remained unchanged at about 700 kg/ha and 600 kg/ha, respectively. No changes in any other measured parameter occurred in response to flower removal. It was feasible to conduct at least three flower harvests starting in mid August and still obtain maximum seed and oil yield. It has been reported that when flowers are picked by hand, up to seven harvests are possible with a yield of 1.7 tonne of dried flowerheads per hectare. The results of this study indicate that the cultivation of calendula for flowers for the botanical market can coexist with the cultivation of calendula as an industrial oilseed crop for the industrial chemicals market.

### **Oil Content and Calendic Acid Levels**

'Resina' seed contained higher percentage of oil at both locations in both years than 'EO',  $15.3 \pm 0.9$  % and  $13.5 \pm 0.8$  %, respectively. Harvested seed was of mixed maturity due to the indeterminate growth habit of calendula. Removal of visibly immature seed (~10% of sample weight) by hand prior to oil extraction resulted in seed oil content increase of 1–2 %.

Calendic acid is the predominant fatty acid in calendula seed. Fatty acid analysis of randomly sampled single seeds showed a large range in calendic acid levels from a low of 38 % to a high of 61 %, on average 53–55 %. Other fatty acids included: linoleic C18:2 (28–43 %, average 35 %), palmitic C16:0 (3.0–6.5 %, average 4.0), oleic C18:1 (3.1–5.5, average 3.5 %), stearic C18:0 (1.5–4.0 %, average 2.0 %), and linolenic C18:3<sup>A9,12,15</sup> (0.5–3.0 %, average 1.5 %). The fatty acid content is within the range reported by other researchers (Beerentrup and Röbbelen, 1987; Breemhaar and Bouman, 1995). No difference in calendic acid content was noted between cultivars.

### **Flavonoid Content**

Flavonoids in calendula are present largely in the form of flavonol glycosides with isorhamnetin and quercetin as main aglycones (Fig. 1). According to literature information flavonols make up 0.3 to 1.5 % of calendula flowers (Vidall-Ollivier et al., 1989). Two main flavonol glycosides detected in both cultivars studied were isorhamnetin-3-rutinoside (I-3-R) and isorhamnetin-3-O-2-rhamnosyl-rutinoside (I-3-RR). The HPLC profile and UV spectra of flavonol glycosides (insert) in calendula flowers is shown in Fig. 2. Quercetin glycosides were not well resolved under the HPLC conditions applied and could not be quantified.

Synthesis of flavonol glycosides was variety and year dependent, and to lesser extent site dependent. Accumulation of secondary metabolites including flavonoids in plants was found to be mainly genetically and environmentally controlled, and to a limited extent by plant nutrition. Over two years of study the levels of flavonols in 'Resina' were in the range of 0.6 to 1.0 %. 'Erfurter' contained higher amount of flavonols than 'Resina' at Saskatoon location in both years, on average 1.7 % vs. 1.0 % in 1999, and

0.7 % vs. 0.6 % in 2000. 'Erfurter' is considered a superior variety for phytomedicinal, nutraceutical and dermatological applications of calendula. Its flowers tend to be bigger and consist of higher proportion of ray florets, which are believed to be main accumulation sites for flavonoids. Spectrophotometric assay used in this study largely underestimated flavonol glycosides content and cannot be recommended for future studies.

The flavonol content in flowers of both varieties decreased from mid August to early September in both years as shown in Fig. 3. Over the three weeks period flavonol levels decreased on average 25 %. Other cultivars may prove better adapted to the growing conditions in western Canada than the two selected for this study. Additional varieties/accessions received from the International Plant Genetic Resources Institute (IPGRI) at Gatersleben, Germany, were grown in 2002 in Saskatoon for seed multiplication purposes and are planned to be subject to larger field trials in 2003.

## CONCLUSION

Calendula has potential as a new crop for production of both seed and late maturing flowers under dryland farming conditions on the western Canadian prairies. Seed yield improvements could be achieved through reduced seed shattering either through breeding or developing better harvest methods. The feasibility of collecting flowers for the botanical and nutraceutical market without compromising seed yield was demonstrated suggesting that calendula can be grown as a dual purpose crop. 'Resina' was found to be higher seed yielding while 'Erfurter' was higher in flavonoid content. Other cultivars may prove better adapted to the growing conditions in western Canada than the two selected for this study. Larger field trials with larger number of cultivars and application of weed control should be conducted in future to gain a clearer idea of the true yield potential.

## ACKNOWLEDGEMENTS

Thanks to the Saskatchewan Agriculture Development Fund for financial support of this project and to Donna Dunlop, Arin Katrusiak and Doug Hassard for the technical assistance with various chemical analyses.

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## **Tables**

Table 1. Plant height and seed yield of calendula 'Resina' and 'Erfurter Orangefarbigen' (EO) grown at Saskatoon, Saskatchewan and Brooks, Alberta.

Seeding Rate	Saskatoon				Brooks			
	'Resina'		'EO'		'Resina'		'EO'	
	Height (cm)	Yield (kg/ha)	Height (cm)	Yield (kg/ha)	Height (cm)	Yield (kg/ha)	Height (cm)	Yield (kg/ha)
6 kg ha <sup>-1</sup>	60	703	51	586	57	781	62	562
12 kg ha <sup>-1</sup>	63	811	52	696	nd <sup>1</sup>	nd <sup>1</sup>	85	498
LSD (0.05) Yield at Saskatoon			259					
LSD (0.05) Yield at Brooks			91					

<sup>1</sup>nd - Not determined

Table 2. Effect of flower harvest on plant height and seed yield of calendula 'Resina' and 'Erfurter Orangefarbigen' (EO) at Saskatoon and Brooks; seeding rate 6kg/ha.

Flower Harvest Date	'Resina' Saskatoon		'EO' Saskatoon		'EO' Brooks	
	Height (cm)	Yield (kg/ha)	Height (cm)	Yield (kg/ha)	Height (cm)	Yield (kg/ha)
None	58	753	51	511	82	592
16 August	61	672	54	720	80	535
26 August	62	637	48	614	80	471
7 September	58	749	50	498	80	420
LSD (0.05) Yield at Saskatoon			186			
LSD (0.05) Yield at Brooks			121			

Table 3. Effect of variety, year of harvest and location on the flavonol glycosides content in flowers of calendula 'Resina' and 'Erfurter Orangefarbigen' (EO).

Flavonol Glycoside	Flavonol Glycoside Content (%w/w)					
	'EO' Saskatoon		'Resina' Saskatoon		'Resina' Brooks	
	1999	2000	1999	2000	1999	2000
I-3-RR <sup>1</sup>	0.87±0.06	0.43±0.04	0.51±0.07	0.37±0.04	0.48±0.10	0.53±0.04
I-3-R <sup>2</sup>	0.83±0.08	0.29±0.05	0.47±0.06	0.22±0.05	0.30±0.09	0.34±0.04
I-3-G <sup>3</sup>	0.03±0.00	0.01±0.01	0.04±0.00	0.03±0.00	0.04±0.01	0.05±0.00
Total	1.73±0.08	0.73±0.05	1.02±0.07	0.62±0.05	0.82±0.10	0.92±0.04

<sup>1</sup>Isorhamnetin-3-O-2-rhamnosyl-rutinoside (I-3-RR)

<sup>2</sup>Isorhamnetin-3-rutinoside (I-3-R)

<sup>3</sup>Isorhamnetin-3-glucoside (I-3-G)

## Figures

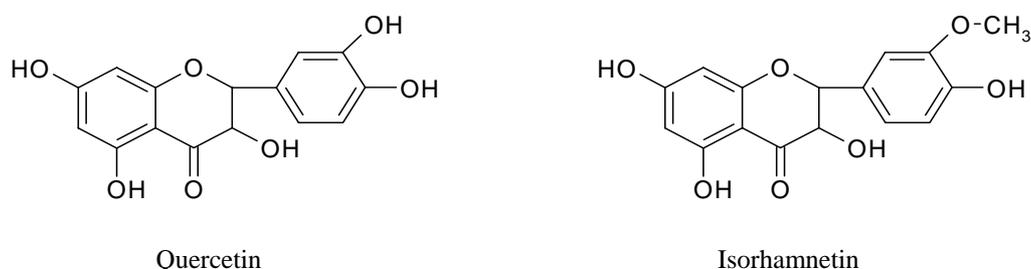


Fig. 1. Chemical structure of two main flavonol aglycons present in calendula flowers.

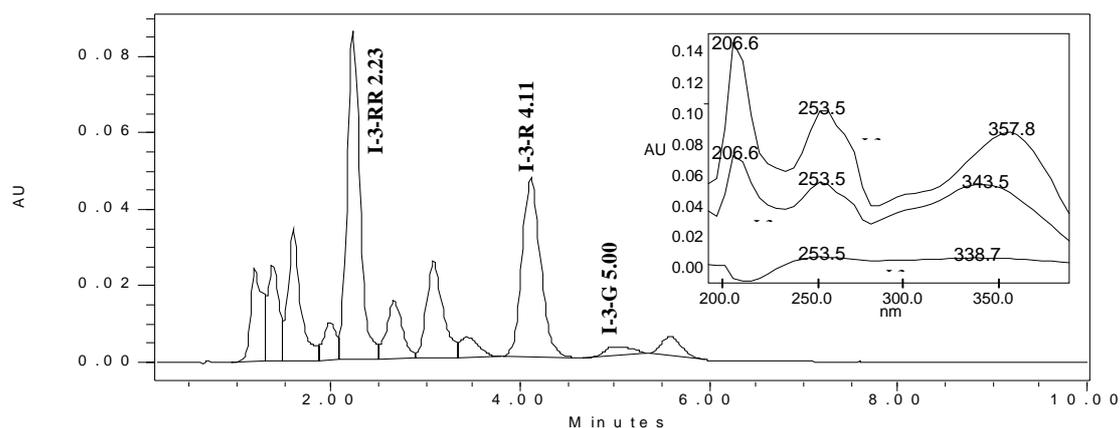


Fig. 2. HPLC profile and UV spectra of flavonol glycosides (insert) in calendula flowers (I-3-RR = isorhamnetin-3-rhamnosylrutinoside; I-3-R = isorhamnetin-3-rutinoside; I-3-G = isorhamnetin-3-glucoside).

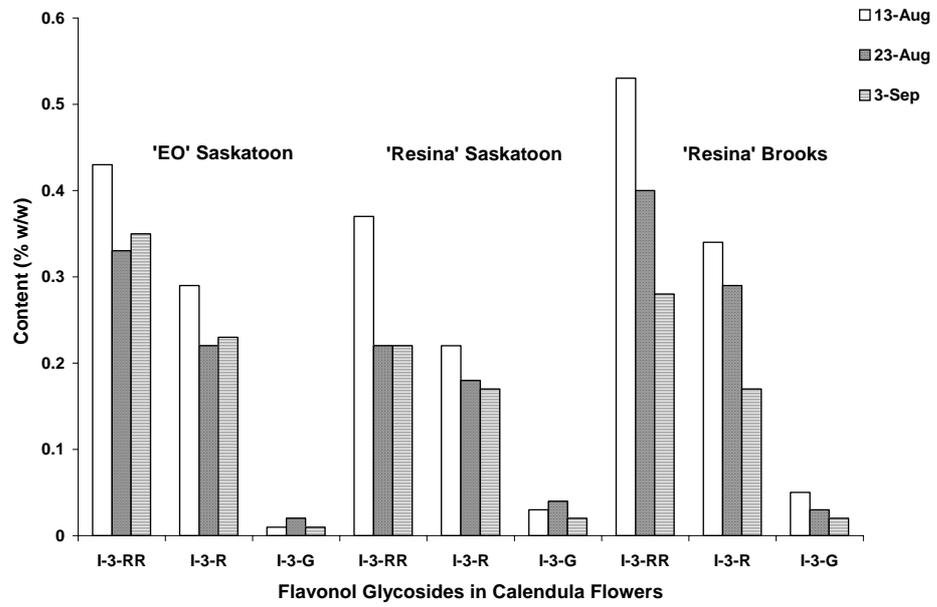


Fig. 3. Effect of harvest time on flavonol glycosides content in calendula flowers (I-3-RR = isorhamnetin-3-rhamnosylrutinoside; I-3-R = isorhamnetin-3-rutinoside; I-3-G = isorhamnetin-3-glucoside).