# Initial studies on seed oil composition of Calendula and Lunaria

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**Abstract**

Two arable oil seed crops, *Calendula officinalis* (calendula or pot marigold) and *Lunaria annua* (lunaria or honesty), were evaluated for their oil yield and composition in three preliminary trials at Lincoln. Fourteen cultivars of calendula had oil yields of c. 15%, with calendic acid ester levels ranging from 25 to 50%. As the calendula seed heads matured, seed size, oil content and calendic acid content of the oil all increased. Lunaria produced seeds with 30-35% oil, containing 21-44% erucic acid and 6-20% nervonic acid.

***Additional key words:*** *Calendula officinalis* L., calendula, marigold, honesty, *Lunaria annua* L., seed yield, flower yield, calendic acid.

## Introduction

There is considerable worldwide industrial interest in sourcing a range of fatty acids with specific properties from renewable resources, especially plants, for conversion into value-added products such as lubricants, polymer additives or surfactants. Oils from current major oilseed crops contain only a limited range of fatty acids, thus there is interest in other oil seed species to extend the range of fatty acids available from renewable sources.

Calendula (*Calendula officinalis* L.), also known as pot marigold, is an annual or short-lived perennial herb native to southern Europe that produces orange to yellow flowers. It is commonly grown as an ornamental throughout the world and is cultivated in southern and eastern Europe. It is grown for a drug, calendulae flos, obtained from the flowers, particularly the petals (Bisset, 1994). There is also interest in growing calendula seed in New Zealand. The seed has an oil content of 5-20% (Meier zu Beerentrup and Robbelen, 1987; Angelini *et al.*, 1997; Cromack and Smith, 1998), of which up to 60% is calendic acid – (8-*trans*, 10-*trans*, 12-*cis*)-octadecatrienoic acid) (Angelini *et al.,* 1997; Cromack and Smith, 1998). This seed oil has similar properties to tung oil and, therefore, could be used as a binder in paints, coatings and cosmetics (Muuse *et al.*, 1992). A previous trial (Martin and Deo, 2000) looked at agronomic production of calendula, but oil content of the seed was not measured.

*Lunaria annua* L. is a member of the Crucifer family, native to south eastern Europe. It is grown in many temperate countries for its translucent fruit, which are ornamental when dried, and is variously known as Honesty, Money Plant, Moonwort or Satin Flower. It is a biennial and has a cold temperature vernalisation requirement (Pierik, 1967). Its seed contains high levels of oil that is rich in long chain erucic and nervonic acids (Princen and Rothfus, 1984), both suitable as lubricants. Nervonic acid may also have pharmaceutical uses. There are violet to purple and white flowering types of Lunaria. Overseas trials have yielded 1-2 t/ha of Lunaria seed with an oil content of around 30-35%, of which 44% was erucic acid and 23% nervonic acid (Cromack, 1998; Mastebroek and Marvin, 2000).

As an initial step to evaluating these two plants for seed oil production, crops were grown in Canterbury and the oils content and composition analysed.

## Materials and Methods

### *Site Details*

Three field experiments, Calendula Cultivar 2002-3, Calendula Time of Harvest 2003-4 and Lunaria 2002-4, were conducted on a Templeton silt loam overlying sand (New Zealand Soil Bureau, 1968) at the Crop & Food Research Farm at Lincoln in Canterbury (172°29'E 43°39'S).

The average soil fertility status (Quick Test values) of the top 300 mm of the 2002-3 trial area, taken in March 2002 was pH 5.9, Ca 9, K 15, P 21, S 10 , and Mg 14. Soil samples taken on the 2003-4 trial area in May 2003 averaged pH 5.9, Ca 7, K 10, P 17, S 4, and Mg 13. Soil physical characteristics on an adjacent area have been given by Martin *et al*. (1992).

### Calendula cultivar trial 2002-2003

Seeds of thirteen garden cultivars of *Calendula officinalis* were sourced from companies in New Zealand, Australia and United Kingdom. Two semi-commercial lines (NZ1 and NZ2) were obtained from New Zealand sources. All 15 lines were sown on 4 December 2002. A randomised block design was used with 4 replications of single rows of each cultivar. There were 15 cm between the rows and 15cm within the row, and 15 seed were sown per row.

The crop emerged between 15 and 30 December, and was top-dressed with urea (100 kg N/ha) on 30 January. The trial area was irrigated (10-35 mm on 11 occasions) to keep soil moistures above 20% gravimetric soil moisture in the top 15 cm and weeded as required to keep weed competition to a minimum. The plants were in bud on 15 February and in full flower by 1 March. The trial was hand harvested on 30 April.

### Calendula time of harvest trial 2003-4

Two Calendula cultivars (NZ1 and NZ2) were sown in the 2003-04 season at Lincoln. The area received 3 t/ha of 30% potassic super incorporated in the soil prior to the sowing of the trial. To prevent any initial weed growth, the area was sprayed with a pre-emergence herbicide (400 g/litre trifluralin at the rate of 1.5 litres/ha). The trial was set out as a randomised complete block with 6 replicates. The crop was sown on 16 October 2003 with a 9 row Oyjord Drill with rows spaced at 15 cm apart, at a seed rate of 24 kg/ha. The plot length was 10 m with plots separated by a tractor wheel mark. Seeding depth was 1-2 cm, and the crop was covered using light harrows attached to the drill. The crop was irrigated with overhead sprinklers at 2-3 week intervals from November to early March. 100kg/ha N was applied as urea (46% N) on 19 November, just prior to the start of flowering. The urea was irrigated in with 25 mm water on the same day. The crop was hand weeded once prior to full flowering.

Six stages of seed head maturity (from completion of flowering until seed dried and shedding) were identified for sampling purposes to test oil composition. These stages were:

1. Petals totally dried and fallen off and the seed-head formed; whole head still green, and seed still soft.
2. Change of sepal colour to light brown; stamens folding in.
3. Seeds hard to the touch and turning darker brown; sepals all drying out.
4. Whole head light to dark brown; seeds hard and all still closely held together.
5. Seed-head matured, seeds all dark brown and closed together. Sepals totally dry and falling off.
6. All seeds very dry, head starting to shed seed if plant lightly shaken.

Twenty randomly selected heads were harvested from the middle five rows of each plot at each stage of maturity between mid-February and mid-March. Due to severe weed infestation in parts of the trial, only the high seeding plus nitrogen treatments within cultivars in two replicates were sampled from the trial.

Seed heads were dried at 30 °C in an airflow drier before manually extracting the seed. Seeds in ten-gram samples of seed from each maturity stage were counted and retained for oil analysis.

**Lunaria Trial 2002-4**

Six different cultivars of *Lunaria annua* seed, sourced from companies in New Zealand, Australia and United Kingdom, were sown at Lincoln on the 4th of December 2002 in a randomized block trial with six cultivars trial replicated 4 times. In each plot, 10 seeds per row were sown by hand at 15cm spacing between and within rows.

There was about 50% emergence over all the lines by 30 January, when 150 kg N/ha was applied as urea. Some plants had to be removed because they showed virus infections, and other plants were damaged by wind or sheep. This reduced plant populations by up to 50% in some plots. The plants were sprayed twice with Orthene before bud development to prevent aphid attack. Buds developed in late September and the plants were all flowering by 20 October. Seed pods formed by 24 November and the trial was harvested on 15 January.

**Oil yields and composition**

All samples were dried at 30 °C and ground. Moisture levels were determined on at least 2 g ground samples on a Sartorius moisture balance using automatic end-point detection. Duplicate samples were extracted for 6 hours with hexane in Stubb extractors. The hexane was removed under vacuum at 50 °C. The oil was dried overnight in an oven at 70 °C and then weighed to determine oil yield.

Fatty acid methyl esters (FAMEs) were prepared from each duplicate oil sample by reacting 5 µl of oil with 100 µl of 0.5M sodium methoxide for 20 minutes at 30 °C. 400 µl of *iso*-propyl alcohol-*iso*-octane (1:9 v/v) were added and left for 20 minutes at 30 °C with occasional mixing to allow the FAMEs to partition into the *iso*-propyl alcohol-*iso*-octane phase. 250 µl samples of the *iso*-propyl alcohol-*iso*-octane containing the FAMEs were removed to vials for immediate analysis by HP 6890 gas chromatograph, using the following operating conditions:

Column – 30 m, 0.32 mm i.d., 0.25 µm film, HP Innowax fused silica capillary column

Injection – splitless mode, automatic injector

Carrier gas – hydrogen, constant flow, 60 cm/sec

Temperatures (°C) – inlet 230, detector 250, oven 150 initial to 190 at 4/minute, then to 230 at 10/minute.

Peak detection was determined by flame ionisation detector. FAME levels are expressed as peak area percentages without correction for detector response. Methyl palmitate, stearate, oleate, linoleate, α- and γ-linolenates and calendate were identified by GCMS. Methyl myristate arachidate, behenate, erucate and lignocerate were identified by comparison of retention times with a commercial AOCS#3 set of FAME standards (American Society of Oil Chemists, 1999).

The results were analysed with analysis of variance using the GenStat statistical package (GenStat Committee, 2003). The time of harvest trial was analysed as a split plot with cultivars as main plots and harvest date as subplots.

## Results

**Calendula 2002-3**

The major constituents of the extracted oil from the 14 lines of calendula seed in the 2002-3 trial are detailed in Table 1. Calendate (25-41%) and linoleate (23-36%) were the major constituents, with myristate (1-11%) and palmitate (7-12%) also quite high in some lines. Minor constituents of the seed oil (i.e. averaging less than 2%) were stearate, oleate, linolenate, arachidate and erucate. Two lines UK8 and UK9 appeared to have more

myristate and palmitate and less calendate and linoleate than the other 13 lines.

Arachidate, lignocerate, myristate, palmitate and stearate are saturated fatty acid esters, oleate and erucate are mono-unsaturated, and calendate, linoleate and linolenate are poly-unsaturated. Table 1 shows very little mono-unsaturated esters in the calendula lines (under 5%). Most lines had under 15% saturated and over 70% poly-unsaturated esters. The exceptions were UK8 and UK9, both of which had around 25% saturated and 55% poly-unsaturated esters.

**Calendula 2003-4**

Thousand seed weight (TSW) increased three-fold up to stage 4 (maturity) ($P<0.05$), and continued to increase from stage 4 to stage 6 (Table 2). Seed oil content also increased considerably ($P<0.05$) with time in both lines, but NZ2 had significantly ($P<0.05$) higher seed oil at all stages than NZ1. As a consequence the seed oil yield increased 10 fold from stage 1 to stages 5 and 6, and was consistently higher in NZ2 at all stages, and significantly so ($P<0.05$) from stage 2 onwards.

**Table 1. Seed oil content (%) and percentage of main oil constituents and levels of fatty acid saturation of total seed oil content in different calendula cultivars in 2002-03. Cultivar origins: NZ = New Zealand, OZ = Australia, UK = United Kingdom). Fatty acids listed in order of increasing carbon number. (LSD = least significant difference and d.f. =degrees of freedom of error.)**

| Cultivar ID | Seed oil content (%) | Myristate | Palmitate | Linoleate | Calendate | Saturated | Mono-unsaturated | Poly-unsaturated |
|---|---|---|---|---|---|---|---|---|
| NZ1 | 18.0 | 3.9 | 8.3 | 29.3 | 35.8 | 13.9 | 4.5 | 66.2 |
| NZ2 | 15.5 | 0.8 | 7.4 | 30.4 | 41.4 | 10.6 | 3.3 | 72.7 |
| NZ3 | 17.9 | 1.7 | 6.6 | 35.4 | 38.1 | 10.7 | 1.1 | 75.0 |
| NZ4 | 17.7 | 2.3 | 8.3 | 34.8 | 35.2 | 12.9 | 1.0 | 71.4 |
| OZ | 18.7 | 4.3 | 7.8 | 35.2 | 33.3 | 14.4 | 1.0 | 69.8 |
| UK1 | 19.9 | 2.7 | 7.8 | 34.2 | 36.4 | 12.5 | 1.0 | 72.0 |
| UK2 | 17.1 | 3.9 | 8.6 | 34.2 | 35.3 | 14.7 | 1.4 | 70.8 |
| UK3 | 21.4 | 2.4 | 7.2 | 34.3 | 37.4 | 12.0 | 1.0 | 72.9 |
| UK4 | 18.3 | 1.7 | 7.5 | 33.8 | 38.1 | 11.3 | 1.1 | 73.0 |
| UK5 | 17.7 | 1.4 | 7.9 | 34.4 | 40.1 | 11.8 | 1.4 | 75.9 |
| UK6 | 16.5 | 1.0 | 8.0 | 35.9 | 38.2 | 11.6 | 3.1 | 75.4 |
| UK7 | 18.1 | 1.0 | 7.6 | 33.6 | 39.7 | 11.2 | 3.6 | 74.6 |
| UK8 | 16.2 | 11.2 | 11.9 | 26.9 | 25.2 | 25.7 | 2.5 | 54.7 |
| UK9 | 19.8 | 11.1 | 10.5 | 25.7 | 27.8 | 23.7 | 1.9 | 55.3 |
| LSD (5%, d.f. 13) | * | 2.9 | 2.0 | 3.6 | 4.7 | 4.8 | 2.5 | 8.5 |

* No LSD as whole sample extracted.

**Table 2. The effect of seed head maturity stages (1-6) on Calendula thousand seed weight, oil content (% dwt) and oil yield (mg/seed) in 2003-04.**

| Head maturity stage | TSW (g) | | Seed oil content (% dwt) | | Seed oil yield (mg/seed) | |
|---|---|---|---|---|---|---|
| | NZ1 | NZ2 | NZ1 | NZ2 | NZ1 | NZ2 |
| 1 | 2.85 | 2.48 | 3.61 | 7.68 | 111 | 191 |
| 2 | 5.29 | 5.21 | 6.78 | 12.40 | 376 | 664 |
| 3 | 5.92 | 5.51 | 5.74 | 13.20 | 335 | 695 |
| 4 | 7.66 | 7.05 | 8.52 | 14.66 | 650 | 1043 |
| 5 | 7.94 | 7.83 | 14.95 | 21.00 | 1206 | 1681 |
| 6 | 8.92 | 8.07 | 15.03 | 20.26 | 1384 | 1623 |
| Lsd (5%) (within cultivars, d.f. 10)) | 0.98 | | 2.61 | | 164.1 | |
| Lsd (5%) (between cultivars, d.f. 11)) | 1.20 | | 2.41 | | 150.7 | |

**Table 3. The effect of seed head maturity stages (1-6) on percentage of Calendula main oil constituents and levels of fatty acid saturation in 2003-4.**

| Head maturity stage | Palmitate | | Oleate | | Linoleate | | Calendate | | Saturated | | Mono-unsaturated | | Poly-unsaturated | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NZ1 | NZ2 | NZ1 | NZ2 | NZ1 | NZ2 | NZ1 | NZ2 | NZ1 | NZ2 | NZ1 | NZ2 | NZ1 | NZ2 |
| 1 | 12.6 | 13.9 | 9.7 | 3.9 | 32.5 | 37.4 | 34.2 | 30.8 | 16.5 | 16.8 | 10.8 | 4.6 | 69.1 | 71.3 |
| 2 | 9.3 | 11.3 | 11.4 | 7.3 | 34.9 | 38.5 | 34.6 | 31.2 | 12.4 | 14.0 | 12.6 | 8.0 | 71.6 | 72.1 |
| 3 | 7.1 | 10.1 | 11.1 | 6.4 | 31.2 | 39.5 | 40.1 | 34.8 | 10.4 | 12.8 | 12.9 | 7.0 | 73.4 | 76.3 |
| 4 | 6.4 | 8.7 | 9.6 | 5.8 | 32.7 | 39.3 | 41.1 | 36.2 | 9.6 | 11.2 | 11.5 | 6.9 | 75.4 | 76.9 |
| 5 | 4.5 | 7.9 | 5.3 | 6.3 | 30.7 | 37.6 | 49.7 | 40.1 | 7.2 | 10.2 | 7.6 | 7.0 | 81.3 | 79.1 |
| 6 | 4.8 | 7.4 | 3.7 | 4.5 | 31.8 | 37.5 | 50.1 | 43.0 | 7.2 | 9.8 | 6.1 | 5.2 | 82.8 | 81.7 |
| LSD (5%) within cultivars, d.f. 10) | 2.28 | | 4.24 | | 2.25 | | 5.87 | | 2.09 | | 4.18 | | 6.49 | |
| LSD (5%) between cultivars, d.f. 11) | 2.09 | | 7.34 | | 24.74 | | 5.76 | | 2.01 | | 8.18 | | 17.08 | |

There were differences in composition of the oil both between stages and between cultivars (Table 3). Calendate increased with increasing seed maturity in both lines, from 34% to 50% in NZ1, and from 31% to 43% in NZ2. There was very little change in linoleate levels, which averaged 32% for NZ1 and 38% for NZ2. Oleate levels did vary with increasing maturity, being highest at Stages 2 to 4 (11% in NZ1, 6% in NZ2), but were only 4% in both lines by stage 6. Palmitate levels decreased with increasing maturity, from 13% to 5% in NZ1, and 14% to 7% in NZ2. Minor constituents of the seed oil (i.e. less than 2%) were stearate, myristate, linolenate, arachidate and erucate.

As a result of these changes, poly-unsaturated oils increased from around 70% to 82% for both lines (Table 3). Both saturated and mono-unsaturated oil content decreased in NZ1, whereas only the saturated oil content decreased in NZ2.

Lines NZ1 and NZ2 were grown in both trials and the oil content and major oil components are presented in Table 4 for the harvested seed in 2002-03, and the seed harvested at head maturity stage 6 in 2003-04. There were large differences in seed oil content between the two seasons, with NZ1 having a lower content, and NZ2 a higher content in 2003-04 compared to 2002-03. There were also large differences in the percentage of oil constituents between the two seasons, especially calendate in NZ1 and linoleate in NZ2.

**Table 4. Seed oil content and percentage of main oil constituents of NZ1 and NZ2 at seed harvest in 2002-03 and at head maturity stage 6 in 2003-4.**

| | NZ1 | | NZ2 | |
|---|---|---|---|---|
| | 2002-03 | 2003-04 | 2002-03 | 2003-04 |
| Seed oil content (%) | 18.0 | 15.0 | 15.5 | 20.3 |
| Myristate | 3.9 | 2.1 | 0.8 | 0.6 |
| Palmitate | 8.3 | 4.8 | 7.4 | 7.4 |
| Oleate | 2.1 | 3.7 | 0.6 | 4.5 |
| Linoleate | 29.3 | 31.8 | 30.4 | 37.5 |
| Calendate | 35.8 | 50.1 | 41.4 | 43.0 |

**Table 5. Thousand seed weight (TSW), seed oil content (%) and percentage of main oil constituents in different Lunaria cultivars. Cultivar origins: NZ = New Zealand, OZ = Australia, UK = United Kingdom).**

| Cultivar | TSW | Oil% | Palmitate | Oleate | Linoleate | Erucate | Nervonate |
|---|---|---|---|---|---|---|---|
| NZ | 15.8 | 21.6 | 3.1 | 28.7 | 8.0 | 36.3 | 18.6 |
| OZ | 16.1 | 23.5 | 2.1 | 24.5 | 6.1 | 44.0 | 18.2 |
| UK1 | 16.3 | 29.7 | 3.1 | 32.8 | 7.1 | 36.4 | 15.2 |
| UK2 | 19.1 | 25.8 | 3.5 | 55.8 | 10.1 | 21.2 | 6.3 |
| UK3 | 16.6 | 24.9 | 2.6 | 28.6 | 7.4 | 37.5 | 19.7 |
| UK4 | 14.3 | 24.5 | 2.4 | 32.1 | 4.9 | 39.2 | 18.6 |
| LSD (5%, d.f. 5) | 2.44 | 13.46 | 0.77 | 4.24 | 1.00 | 5.03 | 2.59 |

***Lunaria***

TSW and seed oil content of Lunaria was very high (Table 5), up to 19 g for TSW and 30% for seed oil content. The major fatty acid esters in Lunaria were erucate (21-44%) and oleate (25-56%), with minor amounts of linoleate (5-10%), palmitate (2-4%) and nervonate (6-20%). Stearate, linoleate, arachidate, behenate and lignocerate were also present in small amounts (under 2% of the total). UK2 had the highest seed weight and highest linoleate and oleate levels, but much lower erucate and nervonate levels compared to the other lines.

## Discussion

Overseas reports present calendula oil contents of 5-20% (Meier zu Beerentrup and Robbelen, 1987; Angelini *et al.*, 1997; Cromack and Smith, 1998), of which up to 60% is calendic acid (Angelini *et al.*, 1997; Cromack and Smith, 1998). The oil contents achieved in these trials was over 15% at maturity for all lines, but calendic acid ester levels ranged from 25 to 50%. However, in both trials, there was considerable variation in oil yield and composition between lines, a feature previously noted by Angelini *et al.* (1997). This indicates that plant breeding could make significant improvements in both oil yields and composition in calendula.

There were also major and inconsistent differences in oil content and composition between the two seasons in the two cultivars NZ1 and NZ2. This may be a consequence of the limited number of samples used for oil analysis in these trials, or of different responses of the two cultivars to environmental factors. For an oilseed industry to be able to produce consistent products, such large differences between seasons would need to be either minimized or predictable.

There is a large increase in seed size and oil content with increasing maturity, coupled with an increase in the proportion that is calendic acid. If oil quality and price is determined by calendic acid content, then calendula seed should not be harvested before stage 5, i.e. seed-head matured, dark brown and closed together, with the sepals totally dry and falling off. At this stage, we have successfully direct headed the crop, without the need for desiccation or windrowing, both of which appear necessary in Europe (Breemhaar and Bouman, 1995).

Calendula is easy to grow in New Zealand (Martin and Deo, 2000) and has been grown on a commercial scale for both petal and seed production. The seed crop has been successfully headed on a field scale. However, current markets for these products are limited, and farmers will need firm contracts before growing this crop.

Overseas trials have yielded 1-2 t/ha of lunaria seed with an oil content of around 30-35%, of which 44% was erucic acid and 23% nervonic acid (Cromack, 1998; Mastebroek and Marvin, 2000). Our trial has lower oil contents (20-30% for most lines), with 21-44% erucic acid and 6-20% nervonate. Also, our oleic acid levels were higher than the 22-25% reported by Walker *et al.*, (2003). However, the lines we used in this trial were commercial flower lines, and there is considerable opportunity to select for high oil lines with the right constituent oils (Masterbroek and Marvin, 2000). It has been suggested that up to 2 t/ha of seed should be commercially achievable in Scotland (Walker *et al.*, 2003), although the crop would take 15 months from sowing to harvest, because the plants appear to need to be of a certain size to be successfully vernalised in winter (Cromack, 1998). Considerable agronomic work will be required to both improve establishment in the field and manage the crop to get high seed yields before Lunaria can be considered a feasible crop for New Zealand's arable farmers.

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