



# Growth and yield response of calendula (*Calendula officinalis*) to sowing date in the northern U.S.

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## ABSTRACT

Calendula (*Calendula officinalis* L.) seed is a rich source of the conjugated C18:3 fatty acid calendic acid and can serve as a replacement for volatile organic compounds in many industrial chemicals such as paints, coatings and adhesives. Calendula is widely adapted to temperate climates and may be a beneficial rotational crop for the northern U.S. where crop diversity is lacking, while potentially providing producers with a new economic opportunity. However, very little is known about its agronomic potential for the U.S. or best management practices for its production. Therefore, a two-year study was conducted in west central Minnesota to evaluate the growth and yield response of calendula to sowing date. One open pollinated calendula cultivar, Carola, and two hybrids, 1557 and 99276, were sown at two-week intervals between early-May and mid-June. Final plant population density was greatest for the early-June sowing (139 plants m<sup>-2</sup>) and declined with earlier or later sowing. Based on the combined analysis of both years, mean seed yield ranged from 1166 to 1839 kg ha<sup>-1</sup> and was greatest for the early-May sowing and declined thereafter. Hybrid 99276 gave the greatest seed yield, which was as high as 2380 kg ha<sup>-1</sup> for the early-May sowing in 2009. Seed oil content averaged 19.4% and did not vary with sowing date, although Carola had slightly greater oil content at 20.5% than hybrids 99276 and 1557, which were 19 and 18.6%, respectively. The number of days from planting to 50% flowering ranged from 52 to 59 d and from planting to harvest about 103 to 115 d. Results indicate that calendula flourishes well in the northern Corn Belt and can be planted and harvested as early as most cold tolerant small grains making it a potentially attractive rotational crop for this region.

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## 1. Introduction

Seeds of calendula (*Calendula officinalis* L.) are a rich source of calendic acid, a conjugated fatty acid (C18:3  $\Delta^{8\text{trans}}, 10\text{trans}, 12\text{cis}$ ) that is highly oxidative. The chemical nature of calendic acid is such that it can be used to replace volatile organic compounds (VOC) as a drying agent in many industrial chemicals including paints, coatings, and adhesives (Muuse et al., 1992; Janssen and Vernooij, 2001; Biermann et al., 2010). Reducing the VOC use in industrial chemicals is a major concern of the European Union and United States.

The seed oil content of calendula is typically in the range of about 16–20% (Meir zu Beerentrup and Röbbelen, 1987; Cromack and Smith, 1998), with calendic acid making up about 55–65% of the total seed oil (Muuse et al., 1992; Biermann et al., 2010). Calendula seed oil can supplement or substitute for tung oil for

some industrial applications. Tung oil is rich in  $\alpha$ -eleostearic acid, which is another conjugated C18:3 fatty acid similar to calendic acid. Tung oil is extracted from seeds of the subtropical tung tree (*Aleurites fordii* Hemsl.) and is almost entirely commercially produced in Southeast Asia. Plant-derived epoxy fatty acids such as vernolic acid can also potentially serve as VOC-free replacements for some of the same industrial uses as calendic and  $\alpha$ -eleostearic acids. Two of the best potential sources of vernolic acid are the seeds of vernonia [*Vernonia galamensis* (Cass.) Less.] and *Euphorbia lagascae* (Spreng.). Vernonia has an exceptionally high seed oil content that ranges from about 35–42% of which as much as 72–80% of this oil is comprised of vernolic acid (Baye et al., 2001). However, vernonia is native to east Africa near the equator, flowers under short days, requires a long growing season and therefore, also is limited to where it can be grown, although advances in breeding have been made (Dierig and Thompson, 1993). An advantage of calendula is that it flourishes in temperate climates, and therefore may be produced over a wider geographical area than tung or vernonia. Additionally, calendula seed oil is relatively high in  $\alpha$ - and  $\gamma$ -tocopherols (Janssen and Vernooij, 2001), which can be used for various industrial and food-use applications.

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Historically, calendula has been grown throughout the world as an ornamental flowering plant and produced commercially in parts of Europe for pharmaceutical uses (Martin and Deo, 2000). Essential oil extracted from calendula flowers has been shown to possess anti-inflammatory properties (Della Loggia et al., 1994) and thus, is used for that purpose in topical burn and wound healing ointments (Janssen and Vernooij, 2001). However, within the past two decades, considerable attention has been given to the development of calendula for the commercial production of its seed oil. Certainly, part of the drive for this comes from societal and government concerns over finding renewable and environmentally friendly replacements for VOCs in industrial chemicals. Some of the agronomic issues with large scale production of calendula have been its susceptibility to seed shattering, indeterminate growth habit, heterogeneity of seed size and shape, and generally low seed yield and oil content (Meir zu Beerentrop and Röbbelen, 1987; Breemhaar and Bouman, 1995; Cromack and Smith, 1998). However, through crop improvement research that has been done in the Netherlands and U.K., including breeding work and crop management, some of these deficiency either have been or are being overcome so that commercial scale production is feasible (van Loo, 2000; Cromack and Smith, 1998). Recently, a study was reported that determined optimum seeding depth for calendula in the northern U.S. (Joly et al., 2013). Nevertheless, more research is needed to characterize the agronomic potential of promising calendula cultivars for various agricultural regions and improve production practices.

Based on research conducted in cool temperate climates (Breemhaar and Bouman, 1995; Cromack and Smith, 1998), calendula may be well suited for production in the northern U.S., especially the northern Corn Belt region. Crop production in the U.S. Corn Belt region is dominated by corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.], and farmers are in need of “third crops” to help diversify crop rotations while adding new economic benefits. Very little is known about the agronomic potential of calendula for the northern U.S. Therefore, the objective of this initial study was to evaluate the optimum seeding date and yield potential of one open pollinated and two hybrid calendula genotypes in west central Minnesota.

## 2. Materials and methods

### 2.1. Cultural practices

The present study was conducted in 2008 and 2009 on a Barnes loam soil (fine-loamy, mixed, superactive, frigid Calcic Hapludoll) at the USDA-ARS Swan Lake Research Farm located 24 km northeast of Morris, Minnesota (45°35'N, 95°54'W). The experimental design was a split-plot randomized complete block replicated four times. The main plots were planting date and subplots consisted of cultivar. Individual plot size was 1.8 m × 7.6 m in 2008 and increased to 3.7 m × 7.6 m in 2009. The three cultivars used in the study were Carola, an open pollinated variety, and 1557 and 99276, which are both hybrids that were developed at Wageningen University in The Netherlands.

Calendula was sown in mid-May (M-M) on 15 May in 2008 and 14 May in 2009, in late-May to early-June (E-J) on 1 June in 2008 and 29 May in 2009, and mid-June (M-J) on 10 June in 2008 and 15 June in 2009. Additionally, an early-May (E-M; 4 May) sowing was made in 2009. The previous crop both years was spring wheat (*Triticum aestivum* L.) and the seed bed for calendula was prepared by fall chisel plowing and lightly harrowing in the spring. Prior to sowing, a fertilizer application of N-P-K-S at 90-34-45-34 kg ha<sup>-1</sup> was incorporated into the soil along with trifluralin at a rate of

1.1 kg ai ha<sup>-1</sup>, in 2008, and pendimethalin at a rate of 1.6 kg ai ha<sup>-1</sup>, in 2009, to control weeds. Both trifluralin and pendimethalin have been found safe to use on calendula (Cromack and Smith, 1998). Seeds were sown with a plot drill at 11 kg ha<sup>-1</sup> and approximately 8 mm deep on 30 cm spaced rows; there were 6 rows per plot in 2008 and 12 in 2009. Calendula seeds used for the study were of the annular morphology (Ruiz De Clavijo, 2005) and seeding and fertilizer management was based on recommendations of Froment et al. (2003).

### 2.2. Plant sampling and measurements

After sowing, a 1 m strip from each of three rows near the center of each plot was randomly selected and marked and later these were used for measuring plant emergence, date of 50% flowering, and final plant population at harvest. Because of calendula's indeterminate growth, when approximately 80% of seed heads (i.e., capitulum) were fully mature (dried and brownish in color), an application of sodium chlorate at 56 g ai L<sup>-1</sup> was made to desiccate plants prior to harvesting as recommended by Froment et al. (2003). Desiccation dates in 2008 were 29 August, 6 September, and 15 September for the M-M, E-J, and M-J sowings, respectively. In 2009, the E-M through M-J sowings were desiccated 12 August, 25 August, 14 September, and 23 September, respectively. Plants were harvested 6 to 7 d after desiccation. In 2008, seed yield was determined by hand harvesting 3 m from one of the center rows. The plants were sealed in mesh bags and further dried in a forced air oven at 45 °C for at least 48 h before threshing and screen cleaning seed. These same plants were used for determining total biomass and harvest index. In 2009, plot width was increased so that plants from the five center rows of each plot could be machine harvested with a plot combine. Moisture content of seed was determined by drying a subsample at 65 °C for 48 h and seed yields were adjusted to moisture content of 10%. Additionally in 2009, plants were sampled from a 1 m strip of row outside the harvest area to determine height, capitulum diameter as well as determine biomass and harvest index after drying and threshing plants. Air temperature and precipitation data were collected at a permanent weather station located at the study site.

Seed oil content was measured by pulsed NMR (Bruker Minispec pc120, Bruker, The Woodlands, TX) as previously described by Gesch et al. (2005). A subsample of approximately 2 g of seed of each replicate was used for analysis. Moisture content was determined according to AOCS (American Oil Chemist's Society) Method 2-75. Each sample was done in duplicate, dried at 130 °C for 4 h, and cooled in a desiccator for 15 min before oil analysis.

### 2.3. Statistical analysis

Data were analyzed by ANOVA using the Mixed Procedure of SAS (SAS for Windows 9.1, SAS Inst., Cary, NC). Trends in data were similar between years, and therefore, combined for analysis. For the Mixed model, sowing date and cultivar were treated as fixed effects and year, replication, year by replication, and sowing date by replication were treated as random effects. Because there was no E-M sowing date in 2008, data were analyzed by both including and excluding the E-M sowing date from 2009. ANOVA results in Table 2 are shown for the analysis excluding the 2009 E-M sowing, but when there was a significant sowing date and/or cultivar effect results are shown including the comparison of E-M for 2009. Mean comparisons were made by least significant difference (LSD) at the  $P \leq 0.05$  level.

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