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Agronomic performance of the novel oilseed crop *Centrapalus pauciflorus* in southwestern Ontario

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ABSTRACT

Centrapalus pauciflorus naturally produces vernolic acid, which could replace the synthetic vernolic acid currently used as a plasticizer. Field trials conducted at Simcoe, Ontario from 2014 to 2016 show two breeding lines of *C. pauciflorus* (PI 642418, PI 642419), performed well under field conditions when grown from transplants. Low field germination rates made agronomic studies using direct seeded plants difficult. Variations in seeding depth, supplemental irrigation, treatment with gibberellic acid and/or fungicide, or priming with water prior to seeding did not significantly improve germination of field sown seed. In general, seed yield, oil content and vernolic acid levels were not affected by nitrogen, or early season removal of the apical meristem. Higher planting densities did significantly increase seed yield and hence overall oil yield per hectare. Over the three growing seasons, oil content ranged from 31.6 to 42.2% (ave. 37.5%) and vernolic acid content of 70.2–76.0% (ave. 73.5%). Additional research to improve field germination of seed would greatly facilitate the continued development of *C. pauciflorus* as a commercial crop for the production of vernolic acid.

1. Introduction

Plasticizers are low molecular weight additives that alter the structure and temperature sensitivity of polymers used to produce plastics (Rahman and Brazel, 2004). Currently, esters of phthalic acid (such as phthalates), derived from petroleum feedstocks are used as additives for manufacturing useful plasticizer-based, value-added products such as polyvinyl chlorides (PVCs), cables, wirings, automotive parts and in the packaging of foods (Rahman and Brazel, 2004). Products made from esters of phthalic acid have shown to have negative implications on both the environment and human health (Tickner et al., 2001). Vernolic oil, a natural source of the common industrial plasticizer vernolic acid, has been studied in the past as a potential renewable source of this important epoxy fatty acid (Muturi et al., 1994). Vernolic oil has a lower melting point and lower viscosity than its fully epoxidized soybean and linseed oil counterparts. The oil's low content of volatile organics and its ability to reduce drying time is useful when used as an additive in alkyd-based paints. Moreover, vernolic oil is naturally epoxidized and hence, does not require further chemical treatment (Muturi et al., 1994). Several plants produce the unusual C18 epoxy fatty acid (12,13 epoxy-cis-9-octadecenoic acid), known simply as vernolic acid, within their seeds (Kleiman et al., 1965). Fostering industrial interest in vernolic oil is hindered by a lack of research into oilseed crops that naturally produce vernolic oil. One such plant is *Centrapalus pauciflorus* is an annual, drought tolerant plant found throughout eastern Africa (Baye and Becker, 2005). Historically, *Centrapalus pauciflorus* of the asteraceae family was known as *Vernonia galamensis*, one of six subspecies that is composed of four botanical varieties (Gilbert, 1986). The species *V. galamensis* has since been renamed as *Centrapalus pauciflorus* (Willd.) H.Rob. (Robinson, 1999). In current literature, *Centrapalus pauciflorus* and *Vernonia galamensis* are used synonymously. A detailed taxonomic description of the *V. galamensis* complex was done by Gilbert (1986). Briefly, the height of *V. galamensis* is both subspecies and geographically dependent, ranging from 0.2 m to 5 m (Perdue et al., 1986). The number of seed heads, which contain self-fertile, hemaphroditic florets, is also dependent on subspecies and geography as shown in a study by Baye et al. (2001). These authors found the number of primary seed heads ranged from 1 to 41 depending on the accession and cultivation location, with 1000 seed weights ranging from 3.10 to 4.58 g *Centrapalus galamensis* ssp typically flower in response to short day length and so are not suitable for cultivation in most of North America. Day neutal varieties of

Abbreviations: SHRS, Simcoe Horticultural Research Station

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Vernonia galamensis were successfully developed in the early 1990's to facilitate future commercial development of this crop in North America (Thompson et al., 1994). The oil content of *Centrapalus pauciflorus* is between 35 and 42%, 72–80% of which is vernolic acid (Baye et al., 2001; Thompson et al., 1994).

Due to its high vernolic oil content, a limited number of trials to evaluate the agronomic performance of *C. pauciflorus* have been conducted, in South Africa, Eritrea, and as far from its origins as the United States (Shimelis and Hugo, 2011; Mebrahtu et al., 2009; Bhardwaj et al., 2000). In North America, agronomic trials on *C. pauciflorus* have been conducted in Arizona and Virginia (Thompson et al., 1994; Bhardwaj et al., 2000), and Ontario (Chakraborty, 2015a, 2015b; Chakraborty et al., 2016). Although there have been attempts to grow *C. pauciflorus* commercially in places like Ethiopia, these have been unsuccessful (Baye and Oyen, 2016). The primary objective of this study was to evaluate how well *C. pauciflorus* would grow in southwestern Ontario. Agronomic conditions (such as choice of breeding line, seeding date, climate and the use of transplants) can have an impact on germination, flowering rates, seed yield and seed oil content. Results from this study will help determine the potential for growing *C. pauciflorus* in southwestern Ontario and what further work may be required to facilitate the development of this plant into a commercial oilseed crop.

2. Materials and methods

2.1. Experimental sites

Field trials were established at the Simcoe Horticultural Research Station (SHRS), located in Simcoe, Canada (42° 51′N; 80° 16′W). At the SHRS, the soil is characterized as fine sandy and loam, with a pH of 6.7 and an organic matter content of 1.2% (Hohner and Presant, 1988).

2.2. Treatments and experimental design

Three, day neutral accessions for *Centrapalus pauciflorus*, (PI 642418, PI 642419 and PI 642420) were obtained from the USDA germplasm bank. These lines were derived from crosses of a highly self-incompatible, day neutral accession of *V. galamensis* subs. *galamensis* var. *petitiana* with 3 short day flowering paternal lines of *V. galamensis* subs. *galamensis* (Thompson et al., 1994; Dierig et al., 2006). Lines PI 642418, PI 642419 and PI 642420 were selected for the ability to produce mature seed under long day conditions in crop maturity intervals common to the continental United States (Thompson et al., 1994; Dierig et al., 2006). Approximately 100 seeds were supplied by the germplasm bank, but these were several years old and had average germination rates in the greenhouse of between 20 and 40%. These seeds were used to generate plants in the greenhouse starting in the fall of 2013 to provide seed for the 2014 experiments. Trails in subsequent years were done using mature seed collected from the plants grown during the previous season. The experimental design for all agronomic trials was a randomized complete block design with four blocks. Each replicate plot contained three 3–4 m long rows. The intra-row and inter-row spacing was trial dependent and guard rows were planted at the ends of each block to minimize edge effects. Ammonium nitrate was applied at 100 kg actual N ha^{-1} prior to planting and hand weeding was conducted as needed throughout the growing season. Measurements and samples were taken from plants in the centre row of each plot. These plants were harvested into large paper bags and, after air drying, the seeds were collected. Seeds were removed from the flower heads manually and the papus was removed using a DB2001 debearder (Mater Seed Equipment, Corvallis, OR). The seed was then cleaned using a Clipper Office Tester (A.T. Ferrell Company Inc., Bluffton, IN) and a CB-1 column air cleaner (Agriculex, Guelph, ON). Mature seed weights were recorded for each replicate in g per 100 seeds. Days to 50% flowering from sowing in the greenhouse (Table 1), were determined using the date when 50% of the plants in the centre row of each plot were in flower

Table 1
Average days to 50% flowering from seeding in the greenhouse and accumulated growing degree days (GDD, 10 °C baseline) for *Centrapalus pauciflorus* grown from transplants. Within columns, values followed by the same letter are not significantly different at the $P \leq 0.05$ level using Tukey's test for multiple means comparisons.

	Average Days to 50% Flowering		
Line Number	2014	2015	2016
PI 642418	73.0[a]	98.2[a]	93.8[a]
PI 642419	68.7[a]	99.0[a]	96.0[b]
PI 642420	83.3[a]	NA	NA
GDD Range for 50% flowering dates	367.4–508.1 (68-83 days)	350.0-362.0 (98-99 days)	578.7–613.9 (94-96 days)
Accumulated Rainfall (mm) from transplanting to 50% flowering	196.6–264.4	177.2–177.4	57.4

2.3. Agronomic evaluation of breeding lines

2.3.1. Field germination

A randomized complete block design with four blocks and 25 seeds per replicate was used for all germination trails. Seed was primed by soaking in distilled water for 24 or 48 h or in distilled water containing 0.05 or 0.2% (w/v) Gibberellic acid (90% GA_3, Acros Organics, NJ). Dry seed was used as the control in all priming experiments. In certain experiments, primed seed was dusted with the fungicide Thiram 75 WP (Chemtura Canada Co., Elmira ON), prior to planting. Seed was typically planted to a depth of 1.25 cm in a 1 m row, but the planting depth experiment involved sowing the seed at depths of 0, 0.6, 1.2 and 1.9 cm (0, 0.25, 0.5, 0.75 inches). The effect of irrigation on germination was studied by applying 0, 0.25, 0.5 or 1.0 L H_2O row m^{-1} day^{-1}. Germination counts were recorded weekly for 3 weeks.

2.3.2. Breeding line assessment

Each of the three breeding lines (PI 642418, PI 642419 and PI 642420) was grown from either field sown seed or from greenhouse grown transplants. The experimental design is described in Section 2.2. Plants were grown at an inter-row spacing of 100 cm and an intra-row spacing of 50 cm (2014) or 25 cm (2015 and 2016), and seed yield was measured from 3 plants harvested from the centre row of each plot.

2.3.3. Fertility

Seed for line PI 642418 was used to generate transplants in the greenhouse. The design of the field experiment is described in section 2.2. Ammonium nitrate fertilizer was applied to individual plots at either 0, 50, 100 or 150 kg actual N ha^{-1}. The applied fertilizer was worked into each plot prior to planting. Plants were grown at an inter-row spacing of 75 cm and an intra-row spacing of 50 cm, and seed yield was measured from 3 plants harvested from the centre row of each plot.

2.3.4. Stand density

PI 642419 seed was used to generate transplants in the greenhouse. The design of the field experiment is described in Section 2.2. Plants were grown with the spacings shown in Table 8, and seed yield was measured from 3 plants harvested from the centre row of each plot.

2.3.5. Plant topping

PI 642418 seed was used to generate transplants in the greenhouse. The design of the field experiment is described above. Plants were grown at an inter-row spacing of 100 cm and an intra-row spacing of 50 cm. When plants reached a height of 25 cm, the apical meristem was removed manually using clippers. The control plants were left uncut.