**Early planting dates maximize winter annual field pennycress (*Thlaspi arvense* L.) yield
and oil content**

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Abstract

Pennycress (*Thlaspi arvense* L.), a common winter annual weed species in North America, has received attention in recent years as a potential oilseed feedstock for biofuel production but little is known about best practices for its production as a managed crop. Therefore, the objective of this study was to determine optimum sowing date to maximize pennycress yield, oil content, and crude protein. Four field experiments with 10 unique sowing and harvest dates over 3 crop years were conducted in Morris, MN, USA. Pennycress was no-till seeded from late August to late October at a rate of 6.7 kg ha⁻¹. Seed yield averaged between 99 and 1109 kg ha⁻¹ when sown in late October and early September, respectively, while oil content for the same sowing period averaged between 26.8 and 36.3%, respectively. Yield was not related to in-season environmental variables, such as cumulative precipitation, soil temperature at planting, or accumulated photohydrothermal time. However, oil content was maximized under greater precipitation ($r^2=0.86$), warmer soil temperatures ($r^2=0.62$) and greater photohydrothermal time when modeled at 2, 4, 6, 8, 25, and 50 cm soil depths (between $r^2=0.53$ to $r^2=0.85$). Results indicate that environment conditions favoring a long maturation period increased oil accumulation in seeds. Conversely, a longer growth period reduced seed crude protein. Although pennycress protein is expected to have industrial uses, managing for yield and oil content is preferred. Therefore sowing pennycress in late August through September in the northern Corn Belt will maximize yields and oil content.

Key words:

Biofuels;
Northern Corn Belt;
Environmental properties;
Photohydrothermal time

1. Introduction

Agricultural residues, oilseeds, and dedicated perennial crops are expected to supply a large portion of the sustainable bioenergy feedstock in both the United States and abroad (US Congress, 2007; USDOE, 2011). First generation crops such as corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] comprise the largest input supply for biofuel generation followed by perennial grasses and woody species. Competing end-uses for agricultural products (i.e. human/animal consumption or biofuel generation) raises issues for developing a mature biofuel market. Too much removal of crop stover and residues from first generation feedstocks for biofuel can result in losses of soil organic carbon (SOC), increased wind and water erosion, and decreased soil fertility (Blanco-Canqui and Lal, 2009; Wienhold and Gilley, 2010; Wienhold et al., 2011; Wilhelm et al., 2007). Additionally, significant overlap between existing ethanol plants in the northern Corn Belt (NCB) represents a possible supply constraint (Schmer and Dose, 2014). Therefore, meeting the demands of a growing world population by balancing grain production for human and animal consumption with the biofuel industry will require additional feedstocks and alternative cropping practices to reduce competing end-uses of agricultural products while simultaneously minimizing environmental degradation.

Sustainable temporal intensification of agricultural systems by increasing the number of crops grown within a season (Heaton et al., 2013) has been suggested as a way to simultaneously produce food, feed, fiber, and fuel for multi-end uses. In the NCB, much of the agricultural land is devoid of vegetative cover from late September to late May (Oschner et al., 2010). This fallow period represents an opportunity to intensify agricultural production through incorporation of winter annual crops. Field pennycress (*Thlaspi arvense* L.) is a winter annual oilseed that is being developed as a cash cover crop for double- and relay-cropping with soybean in the NCB

(Johnson et al., 2015) and shows excellent potential for sustainable agricultural intensification as feedstock for advanced biofuels (Shonnard et al., 2010). As a winter cover, pennycress can prevent soil erosion between summer annual crops, improve water quality through sequestering excess soil N and P, provide early-season nutritional needs for pollinating insects, and add profitability to agricultural systems (Ott et al., 2015; Gesch et al., 2014; Eberle et al., 2015).

Pennycress is native to Eurasia, but is found throughout most of temperate North America (Holm et al., 1997; Warwick et al., 2002). Although largely considered an agricultural weed, pennycress is an attractive biofuel feedstock as it can grow in a wide range of habitats and produce up to 150,000 seeds per plant representing a range of 600-1220 L of oil ha⁻¹ (Marek et al., 2008). The oil from its seeds can easily be converted to biodiesel or jet fuel as pennycress has a high content of erucic and **linoleic** acids (32.8 and **22.4 wt %**) **according to** Moser et al. (2009). Additionally, pennycress seed meal has potential to be used as a biofumigant and weed suppressant (Boateng et al., 2010; Hojilla-Evangelista et al., 2013; Johnson et al., 2015; Moser et al., 2009; Phippen and Phippen, 2012; Vaughn et al., 2006). The planting and harvesting of pennycress is compatible with existing farm equipment and is generally harvested from late May to early June in the Corn Belt region (Phippen and Phippen, 2012; Johnson et al., 2015). The early harvest of pennycress enables double- and relay-cropping of soybeans, thus allowing it to be grown without displacing existing agricultural production. Although pennycress is an attractive alternative oilseed offering many beneficial uses, growing recommendations such as sowing date, fertilizer application, etc. are largely unknown. The objective of this research was to determine optimum sowing date to maximize yield, oil content, and crude protein of pennycress in the NCB.

2. Materials and methods

2.1 Plot establishment, yield and oil content

Data from **three** experiments were used for this study which spanned **3** crop-years (2012/2013, 2013/2014, and 2014/2015) and **10** unique planting and harvest dates ranging from late August to late **October** (Table 1). All experiments were carried out at the USDA-ARS Swan Lake Research Farm, Stevens County, Minnesota (45.68 °N, 95.80 °W) on a Barnes silty clay loam (fine-loamy, mixed, superactive, frigid Calcic Hapludolls) soil. The climate of the area is continental temperate with a historic average of 660 millimeters of precipitation, with a mean high temperature of 21°C during the summer, and a mean low temperature of -13°C during the winter (NOAA/NCEI, 2016). Monthly precipitation values and average temperatures during the study period can be found in Table 2. The treatments in all experiments were arranged in a randomized complete block design with four replications with planting date as the main effect. An automated weather station co-located at the study site recorded daily temperature maximum and minimum, total rainfall, bare soil temperature at 5 cm, and mean photosynthetic active radiation (PAR).

Pennycress (**‘Beecher’**) was sown at a rate of 6.7 kg ha⁻¹ following spring wheat (*Triticum aestivum* L.) from late August to late **October** across the **three** experiments on the dates listed in Table 1. Pennycress seeds were sown at a depth of 0.6 cm with 20 cm row spacing with a no-till drill (Marliss) with double disk openers into wheat stubble. Weeds were controlled by applying 1.1 kg active ingredient ha⁻¹ of *N*-(phosphonomethyl) glycine (glyphosate) approximately 3 to 4 weeks prior to planting pennycress in all experiments. Weeds were controlled by hand through the remainder of the experiment. **Fertilizer was** broadcast by hand in the spring soon after the soil had thawed at a rate of 90-34-34 kg ha⁻¹ of N-P-K. Experiment 2

had Paraquat (292 g ai ha⁻¹) applied in 2013, 2 days prior to harvest to facilitate drying. Plot size was 3 x 9 m in **experiment 1**, 6 x 18 m in **experiment 2**, and 3 x 12 m in **experiment 3**.

Seeds were harvested using a plot combine taking a 1.5 m x plot length strip. The collected seeds were cleaned, dried in a forced oven at 65 °C for 48 h and yields were adjusted to a moisture content of **10% (wt./wt.)**. The oil content of the seeds was analyzed by pulsed nuclear magnetic resonance (NMR) (Bruker Minispec cp120, Bruker, The Woodlands, TX, USA) using 6 g of seed. Prior to NMR analysis the pennycress seeds were dried at 130 °C for 3 hours and cooled in a desiccator for 30 min. The NMR instrument was calibrated with pure oil from pennycress, **and values of oil content are reported as percent (dry wt. oil/dry wt. seed)**. The same seed used for oil content analysis was ground to a fine powder and approximately 200 mg was used to measure total N with a Leco ND-2000 combustion analyzer (Leco Corporation, St. Joseph, MI). Seed N content was multiplied by 6.25 to convert to crude protein content (Gesch et al., 2014).

2.2 Modeling thermal time, hydrothermal time and photohydrothermal time

To account for variabilities in rainfall, temperature, and soil moisture between the experiments and growing years, the Soil Temperature Moisture Model (STM²) (Spokas and Forcella, 2009) was used to model thermal time (TT) and hydrothermal time (HTT). **The STM² model uses a simple pedotransfer function to calculate soil temperature and moisture using the inputs of sand, silt, clay, organic matter, and daily temperature and precipitation values with optional inputs for soil bulk density, and soil moisture at field capacity and permanent wilting point (Spokas and Forcella, 2009). The model outputs daily estimated soil temperature and soil moisture values which were then used to determine thermal time, hydrothermal time, and photohydrothermal time over each set of unique sowing and harvest dates.** Thermal time, or

growing degree days, has been used to predict seed emergence and plant development based on accumulation of heat units above a minimum base temperature (T_{base}) and below a maximum ceiling temperature (T_c) (Baskin and Baskin, 1988). For pennycress, the T_{base} was set at -2.5°C and T_c was set at 24°C according to values by Royo-Esnal et al. (2015). Hydrothermal time, introduced by Gummerson (1986), accounts for soil water conditions and accumulation of HTT occurs only when soil moisture potentials (Ψ) are above a threshold soil water required for seed germination and growth (Ψ_{base}). The Ψ_{base} was set to $-1,500$ kPa, which is the generally accepted permanent wilting point of soil. Within the STM² program the soil texture was set at 43.3% sand, 39.1% silt and 17.4% clay, 3.2% organic matter, bulk density of 1.45 g cm^{-3} , soil moisture at -33 kPa was set to 0.30 % and soil moisture at -1500 kPa was set to 0.13 %. These values were based on soil characterization data obtained for Barnes soil in Stevens County, MN from the National Cooperative Soil Survey Characterization Database (2016). All other soil values were left at the default within the program. Soil moisture and temperature were simulated on a daily basis at 0, 2, 4, 8, 25, 50, and 100 cm soil depths.

In order to account for photohydrothermal period (PhHTT), the daily average of PAR was multiplied by HTT values obtained in the STM² model. Average daily PAR measurements take into account not only PAR intensity and cloud cover but day-length as well, with summer average daily PAR being greater than winter average daily PAR due to the incidence of the sun, but also longer photoperiods in the northern hemisphere.

2.3 Statistical analysis

In order to determine the effects of planting date on pennycress yield and seed quality, sowing dates were grouped into broad classes representing planting dates in late August (15-31 August), early September (1-15 September), late September (16-30 September), and late October

(15-31 October). Data analysis was carried out using Proc Glimmix in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Mean separation was carried out using the least significant difference.

Year was considered a random factor, while planting date class was considered a fixed factor.

Regression analysis was used as a means to integrate the variance among experiments and years. Simple regression procedures using SAS Proc REG were used to fit coefficients of determination for yield, oil, and crude protein as affected by planting date, PhHTT, cumulative rainfall in the growing season from planting to harvest, and soil temperature at time of seeding. All statistical analyses were performed at the $p < 0.05$ level of significance.

3. Results and discussion

3.1 Weather

There was variation in precipitation among the three crop years, especially from August through October, when precipitation in 2013 was 80 and 66 mm greater than in 2012 and 2014, respectively (Table 2). The temperatures between the crop years were fairly consistent. However, in 2013 the cumulative HTT and PhHTT were lower indicating soil temperature and moisture were below the base levels during the latter part of the year.

3.2 Seed Yield

The ANOVA results for the interaction of year by planting date class was significant ($p < 0.01$). Although pennycress was adaptable to a wide range of planting dates, there were variabilities within each growing season (Fig. 1a). In general, yields were greatest for experiment 1 when taking into consideration all planting date treatments, as seeding in early September in 2012 resulted in pennycress yields of over 1,100 kg ha⁻¹. Within the 2013/2014 growing season,

yields decreased with later planting day classes, but the opposite was true for the 2014/2015 growing season.

Although differences in yield were observed for planting date classes, regression analysis was not significant ($p=0.15$) for yields as affected by Julian day indicating pennycress has a wide range of planting dates. The lack of a strong relationship between yields and Julian day of planting was primarily due to the variations in yield between planting date classes and years.

Apart from sowing date, soil temperature and moisture has been found to affect pennycress growth and maturation (Best and McIntyre, 1975). For example, pennycress yields decreased in western Illinois as seeding dates progressed beyond September, which was attributed to a decrease in soil temperatures (Phippen et al., 2010). In this study, pennycress yields tended to decrease as the measured bare soil temperature 5 cm below the soil surface at time of planting decreased, however the regression was not significant ($r^2=0.31$, $p=0.12$), but a stronger positive effect of air temperature on yields was found ($r^2=0.64$, $p=0.02$, data not shown). In addition to soil temperature, Johnson et al. (2015) found pennycress yields generally followed rainfall patterns. In the present study, pennycress yields did not increase as cumulative rainfall within the growing season increased ($r^2=0.24$, $p=0.18$). However, the interactive effects of temperature, soil moisture and photosynthetically active radiation may be more important on seed germination, survival, and yield (Courtney, 1967; Hazebroek and Metzger, 1990).

Seedling emergence, especially for non-domesticated plants like pennycress, and subsequent seed yields have been shown to be restricted to soil temperatures that fall between a permissive range (Benech-Arnold et al. 2000). In addition to soil temperature, light and moisture are important for seed germination and plant growth (Benech-Arnold et al., 2000; Courtney, 1967; Forcella et al., 2000). In this study, accounting for light effects with PAR in PhHTT was

only significant at 6 cm soil depths where a positive relationship between PhHTT and pennycress yields was observed (Table 3, $r^2=0.54$, $p=0.04$). Although no relationship was found between PhHTT and pennycress yields for other soil depths, Royo-Esnal et al. (2015) found that when using PhHTT to predict pennycress emergence, which is critical for maximizing yields, it often failed for accessions originating in the USA. This lack of a relationship between pennycress yields and environmental variables may be confounded by pennycress seed dormancy and differences in climatic conditions where the seeds were produced, as reported by Royo-Esnal et al., (2015). However, breeding programs have been developed to domesticate pennycress and reduce dormancy, improve germination and emergence, reduce pod shatter, and increase seed size (Sedbrook et al., 2014), all of which will lead to greater and more consistent pennycress yields. As these factors improve in pennycress, PhHTT may become a more important variable in predicting yield.

3.3 Oil content

Maximizing oil content of pennycress is very important if it is to become a viable feedstock for biofuels. The interaction of year by planting date class was significant ($p=0.02$), with pennycress producing generally greater oil during the 2013/2014 and 2014/2015 growing seasons (Fig. 1b) and the maximum pennycress oil content (36%) was reached with late August sowing dates in these years. These findings echo those of Phippen et al. (2010) who found that pennycress oil content averaged 35% when sown in September, and sharply declined to 30% when sown beyond October 13. Regression analysis indicates a nearly 0.09% loss in oil for each successive day (Julian sowing day) beyond late August (Fig. 2a) ($r^2=0.76$, $p<0.01$).

Although pennycress oil content was not linked to seed yields ($r^2=0.37$, $p=0.11$, data not shown), oil content production was affected by in-season precipitation, soil temperature at time

of sowing (Fig. 2b), and PhHTT (Table 3). Increased precipitation has been shown to increase oil content of winter oilseeds (Pritchard et al., 2000). Precipitation between planting and harvest dates ranged between 253 and 406 mm which corresponded to an oil content between 26 and 36%, respectively (Fig. 2c). Cumulative precipitation alone, accounted for 86% of the variation in oil content. Nearly 63% of the variation in oil content was related to soil temperature at time of sowing (Fig. 2b), where cooler soil temperatures at time of sowing led to lower oil contents compared with warmer temperatures ($r^2=0.62$, $p=0.02$). This is likely due to improved emergence and longer vegetative growth phase with earlier sowing dates (Gesch et al., 2016). Due to the harsh winter conditions of the NCB, planting pennycress when soil temperatures are near the -2.5°C T_{base} will impede pennycress germination and establishment and may reduce growth making plants more prone to winter kill as observed in winter canola (Holman et al., 2011; Wang et al., 2012). Additionally, pennycress planted later in autumn when environmental conditions are less favorable in the NCB, have lower emergence in the fall with a greater percentage of plants emerging in the spring resulting in a shorter life cycle (Gesch et al., 2016). The influence of shorter vegetative period before reproductive growth stages on declining yields and subsequent oil content have been shown for other oilseed crops such as soybean (Pendleton and Hartwig, 1973; Tanner and Hume, 1978, Johnson, 1987).

The positive correlations between oil content and PhHTT at multiple soil depths (Table 3) modeled from planting to harvest, also highlights the importance of not only available soil moisture and temperature throughout the growing season, but also incidence and accumulation of PAR sufficient for a long maturation period to increase oil content (Mailer and Cornish, 1987; Mailer and Pratley, 1990; Pritchard et al., 2000). Earlier planting dates in August through mid-September have over 12 hours of daylight whereas planting dates around late October have less

than 11 hours of daylight. This suggests that plants emerging in early autumn were exposed to a greater amount of accumulated PAR than later emerging ones, which potentially stimulated greater above and belowground vegetative growth prior to winter dormancy. Additionally, longer day light is important during flowering and seed development as light stimulates oil accumulation in seeds by the production of ATP, photosynthetic oxygen, and RuBisCO bypass activation (Baud and Lepiniec, 2010; Browse and Slack, 1985; Rolletschek et al., 2005; Schwender et al., 2004)

3.4 Crude Protein

Crude seed protein varied between years and the interaction of planting date by year was significant ($p<0.01$). Crude protein was greatest (25.3%) for the late October seeding date in 2014 (Fig. 1c). Moreover, crude protein tended to increase with later Julian sowing dates ($r^2=0.70$, $p=0.01$) (Fig. 3a). Soil temperature at time of sowing and cumulative precipitation had a strong negative effect on crude protein (Fig. 3b-c). A negative relationship was also found between crude protein and the accumulation of PhHTT at soil depths ranging from 2 to 8 cm (Table 3).

There is a trade-off between oil content and crude protein in oilseeds. Oil is the major carbon storage form in oilseeds which supply energy during germination and is accumulated during later developmental stages at the expense of starch and protein (Da Silva et al., 1997; Focks and Benning, 1998; Vigeolas et al., 2004). A decreased timespan of vegetative growth (i.e. later sowing dates) equates to less carbon partitioning to oil and greater stores of protein within the seed. Although, pennycress press cake remaining after oil extraction is expected to be a useful source of protein (Hojilla-Evangelista et al., 2015) with greater nutritional quality than canola and soybean meal (Hojilla-Evangelista et al., 2013), the use of pennycress press cake as

livestock feed is not currently recommended due to the high concentrations of glucosinolates which can interfere with organ function (Daxenbichler et al., 1991; Heaney and Fenwick, 1995; Vaughn et al., 2005). It is expected, however, that protein extracted from press cake will have industrial uses such plastic resins (Selling et al., 2013). Nevertheless, managing pennycress for higher oil content with earlier planting dates would be beneficial at the present time to maximize oil for biofuel production.

4. Conclusions

Although pennycress has a wide range of acceptable sowing dates, sowing in August to September was shown to maximize the interaction of yield and oil content in the NCB. The effect of environmental variables, such as cumulative precipitation, soil temperature and PhHTT time were negligible for pennycress yields, but had a strong effect on oil and protein contents. Greater cumulative precipitation, cumulative PhHTT and warmer soil temperatures at time of sowing maximized oil content by providing favorable environmental conditions suitable to a long pennycress vegetation period at the expense of reduced seed crude protein. Although pennycress proteins are expected to have industrial uses, managing for high yields and oil content is recommended by planting in late August to early September in the NCB.

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443

444 Table 1. Sowing dates of pennycress in Morris, MN, USA over the 2012 to 2015 growing
445 seasons.

Experiment	Sowing Date	Harvest Date
1	11 September 2012	12 July 2013
	30 August 2013	30 June 2014
2	29 August 2013	30 June2014
	18 September 2013	3 July 2014
	24 October 2013	14 July 2014
	26 August 2014	24 June 2015
	17 September 2014	29 June 2015
	20 October 2014	8 July 2015
3	18 September 2013	30 June 2014
	17 September 2014	2 July 2015

446

Table 2. Mean monthly temperatures and precipitation during the study period of 2012 to 2015.

	2012/2013		2013/2014		2014/2015	
Month	Average temperature	Cumulative precipitation	Average temperature	Cumulative precipitation	Average temperature	Cumulative precipitation
	°C	mm	°C	mm	°C	mm
August	20.5	55.6	21.5	49.0	20.8	73.4
September	15.5	0.3	17.9	41.9	15.3	16.5
October	7.0	29.2	6.5	74.4	8.4	9.4
November	0.2	16.3	-1.4	2.3	-5.8	7.4
December	-8.3	5.3	-13.9	3.1	-5.7	2.8
January	-10.8	10.7	-14.7	0.3	-8.5	2.0
February	-10.3	0.3	-15.1	0.0	-12.9	0.0
March	-6.8	33.0	-4.1	8.9	0.5	2.5
April	1.8	15.5	4.9	63.5	8.4	20.1
May	13.1	60.5	14.1	88.9	14.3	148.9
June	19.3	179.1	19.9	148.8	20.8	37.8
July	22.2	57.1	20.5	32.5	22.1	73.9

Table 3. Relationship between photohydrothermal time (PhHTT) modeled at several soil depths and pennycress yields, oil content, and crude protein.

Soil depth	Regression equation	RMSE	r^2	p -value
cm				
-----Yield, kg ha ⁻¹ -----				
6	Yield = -579.68 + 0.001(PhHTT)	87.29	0.54	0.04
-----Oil content, g kg ⁻¹ -----				
2	Oil = 2.60 + 0.00005(PhHTT)	1.68	0.85	<0.01
4	Oil = -2.74 + 0.00005(PhHTT)	2.45	0.67	0.01
6	Oil = 1.75 + 0.0005(PhHTT)	2.43	0.68	0.01
8	Oil = 6.38 + 0.00004(PhHTT)	2.09	0.76	<0.01
25	Oil = -3.60 + 0.00005(PhHTT)	2.29	0.71	0.01
50	Oil = 6.38 + 0.00003(PhHTT)	2.94	0.53	0.04
100	Oil = 4.46 + 0.00004(PhHTT)	2.79	0.57	0.03
-----Crude protein, g kg ⁻¹ -----				
2	Protein = 33.96 -0.00002(PhHTT)	0.90	0.71	<0.01
4	Protein = 35.55 -0.00002(PhHTT)	1.13	0.53	0.04
6	Protein = 34.26 -0.00002(PhHTT)	1.09	0.56	0.03
8	Protein = 32.86 -0.00001(PhHTT)	0.95	0.67	0.01

Figure 1. Pennycress a) yields (kg ha^{-1}) calculated at 10% moisture, b) oil content (%), and c) crude protein (%) means ($\pm\text{SE}$) across planting date classes in Morris, MN planted in 2012/2013, 2013/2014, and 2014/2015. Different letters signify statistical differences following least significant difference mean separation at the $p < 0.05$ level. Data from 2013 was collected from Eberle et al. (2015).

Figure 2. Regression models to describe the relationship of pennycress oil content (%) with a) Julian planting days (DOY), b) soil temperature at time of planting (°C), and c) cumulative precipitation (mm) over the crop year from three years at Morris, MN.

Figure 3. Regression models to describe the relationship of pennycress crude protein (%) with a) Julian day of planting (DOY), b) soil temperature at time of planting (°C), and c) cumulative precipitation (mm) over the three crops years at Morris, MN.

Figure 1

Figure 2

Figure 3