HARVEST TIME OPTIMIZATION OF PENNYCRESS FOR USE WITHIN THE CORN-SOYBEAN ROTATION

A Thesis

SUBMITTED TO THE FACULTY OF THE

UNIVERSITY OF MINNESOTA

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE

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March 2019

Dedication

To my mom.

For being my unwaveringly supportive cheerleader when I needed one the most.

Abstract

Opportunities exist in the Upper Midwest to establish crops in the fallow off-season of the corn (*Zea mays* L.)-soybean (*Glycine max* [L.] Merr.) rotation. One option to fill this temporal space is pennycress (*Thlaspi arvense* L.), an emerging cash cover crop. Pennycress is an attractive option for growers because it is a short season, winter annual species that can function as an environmentally beneficial cover crop while providing economic benefit as an industrial feedstock. However, agronomic management practices have not yet been established for pennycress. The purpose of this thesis is to establish when pennycress physiological maturity occurs and determine appropriate harvest timing; measure the effect of desiccant use on pennycress silicle shatter; assess yield and oil trade-offs of double cropping pennycress with soybean; and quantify the economic viability of a pennycress-soybean double-cropped system.

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1.1. SUMMARY

Agriculture in the Upper Midwest of the United States is characterized by a short growing season and unsustainable farming practices including low-diversity cropping systems and high fertilizer inputs. One method to reduce the magnitude of these problems is by integrating a winter annual into the summer-annual-dominant cropping system. For this reason, pennycress (*Thlaspi arvense* L.) has garnered interest in the agricultural community due to its winter annual growth habit and potential for industrial oil production, which makes it an ecologically and economically desirable crop in the Upper Midwest. Despite decades of research focusing on pennycress as an agricultural pest, little is known about its best management practices as an intentionally cultivated crop. The majority of research on this topic has occurred within the past ten years and there are major gaps in knowledge that need to be addressed prior to the widespread integration of pennycress on the landscape. Here I review relevant agronomic research on pennycress as a winter annual crop in the areas of sowing requirements, harvest, seed oil content and quality, cropping strategies, ecological services, and germplasm development. The major points are as following: 1) there is little consensus regarding basic agronomic practices (*i.e.*, seeding rate, row spacing, nutrient requirements, and harvest strategy); 2) pennycress can be integrated into a corn-soybean rotation, but further research on system management is required to maximize crop productivity and oilseed yields; 3) pennycress

provides essential ecosystem services to the landscape in early spring when vegetation is scarce; 4) breeding efforts are required to remove detrimental weedy characteristics, such as silicle dehiscence and high glucosinolate content, from the germplasm. I conclude that pennycress shows great promise as an emergent crop, however, current adoption is limited by a lack of conclusive knowledge regarding management practices and future research is required over a multitude of topics.

1.2. INTRODUCTION

Pennycress, also known as field pennycress, fanweed, French-weed, and stinkweed, is an emerging Brassicaceae (mustard family) oilseed crop that was first identified for production in 1944 as an industrial lubricant (Clopton and Triebold, 1944). Despite its point of origin in Eurasia, naturalized accessions of pennycress have been found throughout temperate regions of the United States and Canada indicating regional adaptation (Holm et al., 1997; Warwick et al., 2002; Phippen and Phippen, 2013). Interest in pennycress as an agricultural crop has been spurred by the demand for alternative land use to mitigate prominent, but unsustainable, farming practices in the Upper Midwestern United States (Heaton et al., 2013). Unlike winter annual crops grown in the Upper Midwest such as cereal rye and wheat, pennycress seed can be pressed for oil and provide near-term economic benefits to growers (Moser et al., 2009a; b; Moser, 2012). While research has been conducted on pennycress as an agricultural pest for decades (Best and McIntyre, 1975; Warwick et al., 2002), earnest development of agronomic management practices for pennycress as a cultivated crop did not begin until its oil was evaluated as a potential biodiesel crop in 2009 (Moser et al., 2009a; b) followed by the sequencing of the genome in 2012 (Dorn et al., 2013). More research is necessary to determine the agronomic best management practices given the recent emergence of pennycress as a crop. The objective of this review was to compile and summarize current agronomic research on pennycress and identify gaps in knowledge for future research.

1.3. GERMPLASM DEVELOPMENT

Pennycress has excellent potential for rapid domestication and genetic improvement (DeHaan et al., 2016). The advantages of pennycress include a small diploid genome, self-fertility, a rapid lifecycle, and a close genetic relationship with the well–studied Brassicaceae species *Arabidopsis thaliana* and canola (*Brassica napus* L.) (Sharma et al., 2007; Sedbrook et al., 2014; Dorn et al., 2015). The small genome size allowed rapid development of a pennycress transcriptome and draft genome that are being used to identify key genes for domestication (Dorn et al., 2013, 2015). Wild collections of pennycress have been made throughout the U.S., Canada, and Europe for the purpose of developing breeding programs. Some accessions are maintained at the USDA North Central Regional Plant Introduction Station in Ames, Iowa. Collections are also maintained at Western Illinois University and at the University of Minnesota and have been screened for agronomic and oil-quality/end use traits (Sedbrook et al., 2014, Altendorf, 2017). The University of Minnesota breeding program identified phenotypic variation for maturity and yield components but did not identify phenotypic variation for traits such as silicle shatter, erucic acid, and glucosinolates (Altendorf, 2017). Further studies showed that genetic diversity in the UMN pennycress collection was limited

(Frels et al., 2017). Therefore, chemical mutagenesis was used to create new genetic variation (Chopra et al. 2018a, unpublished).

Wild pennycress carries three traits that limit pennycress commercialization: silicle shatter at low moisture content, oil with high erucic acid content, and glucosinolates (Sedbrook et al., 2014). A key factor in the development of pennycress as a crop has been improving the germplasm to reduce these undesirable traits. The reduction in erucic acid and glucosinolate content has largely followed the steps laid out by researchers who developed canola from rapeseed (Bell, 1982; Claver et al., 2017). Recently, pennycress lines with reduced erucic acid content were identified in the chemical mutagenesis populations and through the use of CRISPR-Cas9 gene editing (Chopra et al. 2018b, unpublished; McGinn et al. 2018). In addition, pennycress lines with reduced glucosinolates and silicle shatter were identified (Chopra et al. 2018b, unpublished). Combining these traits into elite breeding lines selected for improved germination, early maturity, and increased yield will further facilitate the adoption of pennycress as a cash cover crop.

1.4. SOWING REQUIREMENTS

Optimum planting date for pennycress varies significantly between regions. The majority of research related to pennycress planting date has occurred in the Upper Midwest and there is evidence across growing locations in this region that early September sowing dates correspond to better establishment than late fall or early spring planting dates (Phippen et al., 2010b; Gesch et al., 2016). However, in the Mediterranean climate of Spain, pennycress is not sown until early October, and, in some cases, may not be planted until early November (Royo-Esnal et al., 2015b, 2017; Gesch et al., 2016). In the Upper Midwest, early fall sowing dates correspond to higher soil temperatures, which favored better germination and a longer vegetative growth period prior to winter frost (Phippen et al., 2010b; Royo-Esnal et al., 2017). In comparison to late fall planting dates, early September planting dates also resulted in higher yields and earlier harvest dates (Dose et al., 2017), which is favorable for double cropping (Phippen et al., 2010b).

There is little consensus regarding planting strategy (Table 1-1). In Minnesota alone, seeding rates ranged from 5.5 to 16.8 kg ha^{-1} (Eberle et al., 2015; Johnson et al., 2015, 2017; Gesch et al., 2016; Dose et al., 2017) with little consistency across research studies. Some of the disparity between seeding rates is likely related to the selected wild type lines used, which retain the characteristic of seed dormancy (Baskin and Baskin, 1989; Hazebroek and Metzger, 1990a). A study conducted in western Illinois directly compared three different seeding rates, 1.1, 2.2, and 4.9 kg ha^{-1}, but researchers were not able to draw conclusions regarding optimal planting rate (Phippen et al., 2010a). Seeding method was also evaluated, and it was determined that drilled plots had better stand establishment and higher yields than broadcasted plots regardless of seeding rate (Phippen et al., 2010a). Pennycress is positively photoblastic (Hazebroek and Metzger, 1990b), and a single study concluded that broadcast seeding enhanced pennycress germination due to the increased exposure of seed to light (Carr, 1993). Despite this finding, the majority of recent studies are consistent with the results of Phippen et al. (2010a) and have sowed pennycress at a shallow depth using a seed drill (Table 1-1).

Pennycress is susceptible to environmental influences, and water availability is the major limiting factor to germination and development (Royo-Esnal et al., 2015b; Gesch et al., 2016). Germination occurs most readily in cool, wet conditions (Hazebroek and Metzger, 1990a; b; Johnson et al., 2015; Royo-Esnal et al., 2015b, 2017) and pennycress requires between 25 and 40 mm of water to germinate (Royo-Esnal et al., 2015b; Johnson et al., 2017). Royo-Esnal et al. (2015) noted that only 5% of seedlings emerged when soil moisture levels were low at planting compared to 18% emergence in years with adequate soil moisture. In comparison to sympatric species such as wild oat (*Avena fatua*) and wheat (*Triticum aestivum*), pennycress has low water use efficiency (Anderson and Best, 1965), requiring 405 kg of water to produce 0.45 kg of dry matter (Hazebroek and Metzger, 1990b). Depressed yields are directly associated with limited water availability as low moisture levels can decrease the likelihood of branching (which allows pennycress to compensate for low stand density) (Matthies, 1990), and may reduce pennycress vertical growth to only 1.25% of its potential height (Best and McIntyre, 1975). In general, pennycress is not well adapted for arid environments where natural precipitation is the only form of moisture (Royo-Esnal et al., 2017). Johnson et al. (2017) remedied issues associated with limited moisture by watering pennycress plots with 25 mm of water to promote germination. Another study conducted in Montana showed that when supplemental irrigation was provided, pennycress yielded 1684 kg ha^{-1} (Clopton and Triebold, 1944). Alternatively, higher planting rates can be used to compensate for potentially lower germination rates during dry conditions (Johnson et al., 2015), but may not be a reliable way to meet yield goals. In the future, breeding efforts will be necessary to develop pennycress varieties suitable for dryland regions. While pennycress seeding method and seeding depth have been established, optimal seeding rate over a variety of climatic conditions has not been well developed and requires future research.

Fertilization requirements

Few studies have characterized the direct fertilization needs of pennycress. Ongoing experiments with pennycress within the corn-soybean rotation suggest that no fertilizer application may be necessary for successful production due residual nutrients applied during the summer annual cropping portion of the system. Yield response due to the application of nitrogen and sulfur fertilizers on pennycress seed yield and oil content was quantified by Rukavina et al. (2011). This study, conducted in western Illinois, indicated that a fall application of 112 kg ha^{-1} N produced the maximum seed yield of 995 kg ha^{-1}, but did not significantly outperform treatments with lower fertilizer rates. This indicates that maximum yields may be achieved with fewer resources. The lowest N application rate that maintained the highest pennycress yield required a split application of 28 kg ha^{-1} applied in both the fall and spring (Rukavina et al., 2011). Recent studies in Minnesota have utilized a spring application of 90, 34, and 34 kg ha^{-1} of N, P, and K, respectively, following spring thaw before pennycress reproductive development begins (Eberle et al., 2015; Dose et al., 2017). However, the benefits of fertilization were not quantified. There is also a risk of plant lodging due to over-fertilization, which is exacerbated when pennycress is exposed to extreme weather events (*i.e.*, heavy winds or excessive precipitation) after plants have elongated during reproductive development (personal observation). Overall, there is no clear fertilization strategy to optimize pennycress production and more research is needed on this component of the pennycress production system.

1.5. HARVEST

Current pennycress lines are prone to shatter, which increases susceptibility to seed loss at harvest when plants are at low moisture content (Best and McIntyre, 1975; Sedbrook et al., 2014; Dorn et al., 2015). This loss can be caused by environmental disturbances, such as high wind or precipitation, or by cultural disturbances, such as harvest or planting equipment, which has been noted in related Brassicaceae species (Vera et al., 2007; Sintim et al., 2016). Loss due to shatter directly corresponds to pennycress yield reduction at harvest and can significantly contribute to the weed seed bank as a single plant can shed between 1600 and 15000 seeds (Best and McIntyre, 1975). Carlson et al. (2018) found that seed loss due to shattering was equivalent to 300 times the initial seeding rate. Despite these challenges, it is largely agreed that pennycress is a good candidate for mechanical harvest (Clopton and Triebold, 1944; Eberle et al., 2015; Dose et al., 2017), which is most efficient when seeds are dry and silicles are prone to shatter. One strategy for reducing seed loss due to shatter is to harvest pennycress at physiological maturity. A study by Cubins et al. (unpublished) identified the period when pennycress physiological maturity occurred and, thus, when the crop should be harvested to minimize seed loss due to shattering. This study demonstrated that pennycress physiological maturity occurred nearly two weeks prior to typical harvest periods, when seed moisture was low enough for effective mechanical harvest. Delaying harvest until harvest maturity resulted in a 70% loss in seed compared to harvesting at physiological maturity, *i.e.* maximum yield potential. Consequently, Cubins et al. (unpublished) proposed the use of a harvest aid at physiological maturity to dry pennycress enough to facilitate efficient mechanical harvest. Most agronomic studies reviewed were harvested by hand (Table 1-2), which does not require seeds to have reached full maturity by the time of harvest. In some studies, paraquat dichloride (1'dimethyl-4,4'-bipyridinium dichloride) was applied to assist plant drying prior to combining (Eberle et al., 2015; Dose et al., 2017) and may be used as a desiccant in the future to decrease the time between physiological maturity and harvest maturity. Harvest timing spanned mid-May through mid-July at all reviewed study locations. Studies conducted in Illinois were harvested in late May or early June (Phippen et al., 2010b; Rukavina et al., 2011; Phippen and Phippen, 2012) whereas studies in west central Minnesota and North Dakota were harvested between late June and early July (Carr 1993; Eberle et al. 2015; Gesch et al. 2016; Dose et al. 2017; Cubins et al., unpublished). Differing growing conditions (*e.g.*, length of winter and amount of precipitation) are highly influential in determining when pennycress reaches maturity and can be harvested. Loss of seed at low moisture content in pennycress has been well documented, however, strategies to reduce moisture content when seed reaches physiological maturity to facilitate effective mechanical harvest while reducing shatter loss should be the focus of future research.

Seed yield

The primary product of pennycress production is oil (Moser et al., 2009a; Moser, 2012), and this can be optimized by simultaneously maximizing seed yield and seed oil content. One estimate suggests that pennycress yields need to reach 1684 kg ha^{-1} to be profitable (J. Sedbrook, personal communication, 8 May 2018). While pennycress has potential for prolific yields, studies of current undomesticated lines have demonstrated a high degree of yield variability. Of the studies included in this review (Table 1-2), only two had yields that exceeded the proposed threshold of profitability and each had treatments that yielded significantly lower values (Carr, 1993; Johnson et al., 2017). A high degree of phenotypic plasticity in pennycress partially compensated for low stand density by increasing the seed yield of individual plants in response to decreased population (Matthies, 1990). Due to the branching morphology of pennycress, yields across planting rates were similar to each other at half or a quarter of the maximum seeding rate of 4.9 kg ha^{-1} (Phippen et al., 2010a). While reliance on branching may be a good strategy in years with unexpected stand loss, planting at a higher seeding rate is a more reliable method of increasing stand uniformity and reducing the risk of low yield due to poor germination (Johnson et al., 2015). Overall, there is consensus that pennycress yield is highly dependent on growing season environmental factors (Johnson et al., 2017), which further complicates the designation of pennycress best management practices. Yields in one study reflected rainfall patterns because germination was highly dependent on soil moisture (Johnson et al., 2015). Dose et al. (2017) presented contrary evidence to this finding suggesting that pennycress yield was not correlated to seasonal variables such as precipitation, soil temperature at planting, or cumulative photohydrothermal time, but was most reflective of air temperature at planting in which earlier planting dates corresponded with higher yields. Although environment greatly influences yield variability in current lines of pennycress, planting at higher seeding rates may reduce this and increase stand uniformity.

1.6. SEED OIL CONTENT AND QUALITY

Pennycress seed oil content ranges between 26% and 39%, which makes it acceptable for biodiesel production (Clopton and Triebold, 1944; Moser et al., 2009a; Phippen and Phippen, 2013; Isbell et al., 2015; Gesch et al., 2016; Dose et al., 2017). A range of seed oil content from recent agronomic studies can be found in Table 1-2. Pennycress oil is mainly comprised of components of the oleic acid biosynthetic pathway: linoleic, linolenic, eicosenoic, and erucic acids (Sedbrook et al., 2014). Of these products, erucic acid accounts for the majority of seed oil content and reported values have consistently ranged between 29% and 36% (Isbell 2009; Moser et al. 2009b; Cermak et al. 2013; Phippen and Phippen 2013). Established extrusion procedures have yielded 600 to 1200 L ha^{-1} of pennycress oil (Moser et al., 2009a), which is equivalent to 663 to 1326 kg ha^{-1} of oil. Major components of seed oil quality stabilize prior to seed yield maximization and oil content stabilization (Cubins et al., unpublished).

Similar to seed yield, pennycress oil content is heavily dependent environmental influence, namely precipitation and soil temperature, which both fluctuate greatly with planting date. When pennycress was planted later in the fall, low soil temperatures accounted for 63% of variation in seed oil content and a 0.09% reduction in oil content for each day planting was delayed following late August (Dose et al., 2017). A longer maturation period favored high oil content (Dose et al., 2017), but lower erucic acid levels were associated with later planting dates (Phippen et al., 2010b), which may be due to a shorter growth period. Furthermore, precipitation heavily influenced seed oil content and accounted for 86% of variation in oil yield (Dose et al., 2017). A year with 253 mm versus 406 mm of precipitation yielded seed with 26% and 36% oil, respectively (Dose et al., 2017). Overall, seed oil content was stable across drilled and broadcasted planting methods and was not shown to be as variable as yield over planting method or varied seeding rates (Phippen et al., 2010a). Seed oil content has been relatively stable across research studies and methods, but still experiences variation due to environmental factors.

1.7. CROPPING STRATEGIES

Pennycress's high oil content can be leveraged by including pennycress in an integrated cropping system as a double or relay crop (Clopton and Triebold, 1944; Carr, 1993; Heaton et al., 2013). Due to its early summer harvest, pennycress can be double cropped with soybean and other high value summer annuals without jeopardizing yield of the summer annual crop (Isbell, 2009; Moser, 2012; Phippen and Phippen, 2012; Johnson et al., 2017). In studies with soybean, pennycress increased soybean yield compared to winter-fallow control plots, potentially due to the increased availability of soil moisture (Phippen et al., 2010b; Phippen and Phippen, 2012; Johnson et al., 2015). There were no differences in oil content or protein of soybeans double cropped with pennycress when compared to soybeans grown without a fall-seeded crop (Johnson et al., 2015). Despite a slightly shorter growing season in south central MN, double cropped soybean had ample time to reach maturity before the first fall frost (Johnson et al., 2015). Phippen and Phippen (2012) demonstrated that, when double cropped with soybean in western IL, pennycress had a positive effect on soybean yield in both years of the study, but was only significant in the second year of the study, further indicating the importance of annual environmental fluctuation. Only one study found that pennycress reduced soybean grain yield by 18-30% when double or relay cropped (Johnson et al., 2017). Although there was a reduction in soybean yield, total annual grain yield was increased by 48-102% depending on location and environment when oilseed yields (*i.e.*, pennycress and soybean) were combined (Johnson et al., 2017). Similarly, in a relay cropping study where a full season soybean was no-till planted into standing pennycress, total annual seed yield was 79% greater than a conventionally-managed soybean planted without a relay crop (Ott et al., 2019, in press).

Despite the potential economic benefit of incorporating pennycress into a crop rotation, risks may be incurred. Integrated systems will be most successful in areas with ample precipitation as pennycress yields may be negatively affected by intercrop competition for water (Clopton and Triebold, 1944; Johnson et al., 2015; Royo-Esnal et al., 2017). This has the potential to negate economic benefits if seed yield is not great enough to match input costs. Partly due to its small seed size, pennycress had trouble germinating in high residue environments (Eberle et al., 2015) indicating that planting pennycress into annual crops with a large amount of stubble or leaf litter following harvest may be unsuitable for pennycress production unless residue is removed prior to planting. In short, pennycress can be integrated into a summer annual cropping system while increasing total annual oilseed yield, however, it is not without challenges and, in some years, the risks incurred may be greater than the profit contributed by the winter annual oilseed.

1.8. ECOLOGICAL SERVICES

An ability to scavenge nutrients has made pennycress an attractive option as a low-input winter annual. Between 73 and 77% of annual nitrate loss in the corn-soybean rotation occurs between April and June (Randall and Vetsch, 2005), which suggests excess soil nitrogen availability to support a winter annual crop. Soybean double cropped with pennycress had 18 to 19% less nitrogen in the soil profile down to 60 cm in the fall compared to a fallow plot and 53 to 72% less nitrogen in the spring after oilseed harvest and before soybean planting (Johnson et al., 2017). Similarly, soybean relay cropped with pennycress sequestered between 35 and 40 kg ha^{-1} of nitrogen by mid-spring, which was comparable to a crop of winter rye (*Secale cereale* L.) (Ott et al., 2019, in press). This corresponded to a 20 kg ha^{-1} reduction in soil nitrate levels when directly compared to a fallow tilled control (Ott, 2018) and indicates that pennycress is efficiently able to remove nitrogen from the soil profile.

Pennycress can be utilized as part of a comprehensive integrated weed management strategy (Vaughn et al., 2005; Johnson et al., 2015). Fall establishment can provide early spring ground cover and suppress aggressive spring-germinating weeds such as common lambsquarters (*Chenopodium album* L.), giant ragweed (*Ambrosia trifida* L.), and tall waterhemp (*Amaranthus tuberculatus* L.) (Johnson et al., 2015). Johnson et al. (2015) speculated that weed suppression may have been caused by allelopathic compounds rather than ground cover when pennycress seeding rates and companion crops were considered. The majority of weed suppression research has focused on the use of the sinigrin degradation products allyl isothiocyanate and allyl thiocyanate in pennycress seed meal to suppress weed seed germination (Vaughn et al., 2005, 2006; Isbell, 2009). Pennycress seed meal can provide 100% suppression of weed seeds when incorporated into the soil at 1% (wt/wt) (Isbell, 2009). Similarly, pennycress seed meal was able to suppress the germination of both sicklepod (*Senna obtusifolia* L.) and velvetleaf (*Abutilon theophrasti* L.) at allyl isothiocyanate and allyl thiocyanate concentrations of 5 ppm, and provided over 90% control of weeds in both tarped and untarped field conditions (Vaughn et al., 2005). While acting as an effective weed control, what remains unclear is whether these compounds stay active in the soil and pose risks to intentionally seeded crops if they are planted too soon after pennycress harvest or residue incorporation. Allyl isothiocyanate and allyl thiocyanate were found to suppress the germination of wheat, annual ryegrass (*Lolium multiflorum* L.), and arugula (*Eruca vesicaria ssp. sativa* L.) at 1, 1, and 5 ppm, respectively (Vaughn et al., 2005). Another study found that wheat germination was inhibited by pennycress seed meal at levels as low as 0.1% (Vaughn et al., 2006).

There is also potential for pennycress to be an integral part of an effective and sustainable integrated pest management strategy. Pennycress increased biocontrol potential and provided habitat for spiders, a generalist predator (Groeneveld et al., 2015). Spider abundance was not affected by pennycress growth in a corn-pennycress system and spider species richness and diversity increased when compared to three common corn crop rotations, as characterized by the Shannon index (Groeneveld et al., 2015). The addition of pennycress to a corn rotation also increased and stabilized ground beetle diversity more effectively than a white mustard (*Sinapis alba* L.)-corn rotation, a green fallow-corn rotation, or a bare fallow-corn rotation (Groeneveld and Klein, 2015). This was mainly due to the evenness of plant cover throughout the growing season.

Pollinators can also benefit from pennycress's early flowering time. Forage is scarce in the early spring when most plants on the landscape have not germinated or bloomed, but pennycress can help to fill this gap. In one year of study, pennycress produced only 13 kg ha^{-1} of nectar in comparison to camelina and canola, which produced 100 and 83 kg ha^{-1} of nectar, respectively (Eberle et al., 2015). While pennycress produces less nectar than other angiosperms, as many as 68 insects per minute visited its flowers, indicating that pennycress may provide other resources to pollinators such as pollen. Indeed, pennycress produced 38 kg ha^{-1} of pollen compared to 58 and 109 kg ha^{-1} by camelina and canola (Thom et al., 2018). Recorded visitors mainly consisted of flies, but an earlier flower period over one year of the study lead to a 200% increase in the number of small bees visiting, potentially due to a lack of other flowering resources on the landscape. Research conducted in Germany concluded similarly that the majority of flower visitors were flies, but also noted a number of bee visitors (Groeneveld and Klein, 2014). Pennycress provides services that are imperative to a sustainable ecosystem and the integration of this crop can reduce nutrient leaching, decrease pest pressure, and increase the population of beneficial organisms.

1.9. CONCLUSIONS

Few studies have tackled questions pertaining to pennycress best management practices and many questions remain unanswered. Techniques used in pennycress agronomic research varies greatly among researchers and requires clarification. The time of seeding and seeding method are established parameters, but seeding rate, seeding depth, and row spacing warrant further evaluation to optimize plant density under both semi-arid and humid conditions. Nutrient recommendations are very preliminary, and pennycress's baseline requirements must be quantified. Using this information, researchers will be able to determine whether pennycress requires nutrient input or if its scavenging ability is sufficient without additional fertilization. Techniques to reduce pennycress shatter loss at harvest will also be necessary in ensuring maximum profitability, and breeding strategies have been implemented to develop non-shatter lines for future use (Dorn et al., 2015). Until more shatter resistant cultivars are available for production, refined harvest management strategies are necessary to reduce pennycress moisture to facilitate the earliest harvest after physiological maturity occurs. This will allow pennycress to be mechanically harvested on a field scale and double cropped with corn, soybean, or other high-value summer annuals. Future studies outlining economically important pests (*i.e.*, weeds, insects, nematodes, and diseases) also need to be conducted. Attention to pennycress as a cash cover crop is relatively recent and additional research is required to determine best management practices before large-scale adoption can occur.

1.10. TABLES

Table 1-1. Outline of planting information from recent (2010-2017) pennycress research studies.

Study	Location (USA unless noted)	Treatment investigated	Seeding rate kg ha^{-1}	Planting method	Spacing cm	Seeding depth cm	Fertilization kg ha^{-1} N-P-K
Dose et al. 2017	Swan Lake, MN	Planting date	6.7	Drilled	20	0.6	90-34-34
Eberle et al. 2015	Swan Lake, MN	Pollinator preference	6.7	Drilled	19	1.3	90-34-34
	Elkton, SD			Frost seeded		---	
Gesch et al. 2016	Swan Lake, MN	Effect of seed position	11.6*	Not reported	25	1.0	Not reported
	Teruel, Spain					1.0	
Groeneveld and Kelin 2014	Dundenheim, Germany	Pollinator preference	3.0-5.0	Broadcasted	---	---	Not fertilized
Carr 1993	Guptill, ND	Yield	Not reported	Broadcasted	---	---	Not reported
Johnson et al. 2015 Experiment 1	Lamberton, MN Rosemount, MN Waseca, MN	Companion crop Location Seeding rate	5.5, 11.0	Drilled	25	Not reported	Not reported
Johnson et al. 2015 Experiment 2	Lamberton, MN Rosemount, MN Wasecea, MN	Companion crop, location, seeding date, seeding rate, variety	5.5, 11.0	Drilled	25	Not reported	Not reported
Johnson et al. 2017	Rosemount, MN Saint Paul, MN Waseca, MN	Cropping system, harvest time	16.8	Drilled	10	Not reported	Not reported
Phippen et al. 2010a	Macomb, IL	Planting method, seeding rate	1.1, 2.2, 4.9	Broadcasted, drilled	---, 7.5	---, 1.0	Not reported
Phippen et al. 2010b	Macomb, IL	Herbicide Planting date	Not reported	Drilled	7.5	Not reported	Not reported

Phippen and Phippen 2012	Macomb, IL	Double crop	6.7	Drilled	19	Not reported	Not fertilized
Rukavina et al. 2011	Macomb, IL	Fertilization	4.5	Not reported	---	---	0, 28, 56, 84, 112 N, 11 S
Groeneveld and Klein 2015	Oβweil, Germany	Insect diversity	22	Not reported	---	---	Cattle manure, unknown
Groeneveld et al. 2015	Oβweil, Germany	Insect diversity	22	Not reported	---	---	Cattle manure, unknown

*Average 1000-seed weight for seeds at harvest maturity from Cubins et al., unpublished, used to extrapolate planting rate

Table 1-2. Outline of harvest information from recent (1993-2017) pennycress research studies.

Study	Location (USA unless noted)	Treatment investigated	Harvest method	Minimum yield kg ha^{-1}	Maximum yield kg ha^{-1}	Minimum oil content %	Maximum oil content %
Dose et al. 2017	Swan Lake, MN	Planting date	Plot combine	200	1100	34	36
Eberle et al. 2015	Swan Lake, MN	Pollinator preference	Plot combine	230	1110	Not reported	Not reported
	Elkton, SD		Hand harvest	0	520		
Gesch et al. 2016	Swan Lake, MN Teruel, Spain	Effect of seed position	Hand harvest	Not reported	Not reported	32 26	33 31
Carr 1993	Guptill, ND	Yield	Hand harvest	119	1628	26	26
Johnson et al. 2015 Experiment 1	Lamberton, MN	Companion crop	Hand harvest	104	113	Not reported	Not reported
		Seeding rate		161	168		
	Rosemount, MN	Companion crop, seeding rate		477	1387		
	Waseca, MN	Companion crop		390	513		
		Seeding rate		672	694		
Johnson et al. 2015 Experiment 2	Rosemount, MN (2010/2011)	Companion crop	Hand harvest	133	270	Not reported	Not reported
		Seeding date		116	204		
	Rosemount, MN (2012)	Companion crop		441	1129		
		Seeding date		441	1245		
		Variety		796	1245		
Johnson et al. 2017	Rosemount, MN	Cropping system, harvest time	Hand harvest	500	1000	Not reported	Not reported
	Saint Paul, MN			1450	2400		
	Waseca, MN			75	1200		

Phippen et al. 2010a	Macomb, IL	Planting method, seeding rate	Hand harvest	345	535	28	29
Phippen et al. 2010b	Macomb, IL	Herbicide	Hand harvest	150	1120	30	34
		Planting date		Not reported	Not reported	30	36
Phippen and Phippen 2012	Macomb, IL	Double crop	Not reported	672	896	Not reported	Not reported
Rukavina et al. 2011	Macomb, IL	Fertilization	Hand harvest	351	1117	34	36

2. PENNYCRESS SEED YIELD AND OIL CONTENT AND QUALITY AS CHARACTERIZED BY HARVEST TIMING

2.1. SUMMARY

Corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.) dominate the Upper Midwest landscape, but only for six to seven months of the year. Thus, opportunities exist to establish crops that will utilize the remainder of the short growing season while contributing to farm profitability in a sustainably intensified manner. Field pennycress (*Thlaspi arvense* L.) is a short season winter annual species that has been identified as a feedstock for advanced biofuels that can occupy this space. However, the prominence of pod shatter in pennycress is a significant agronomic barrier to production. My aims were to identify the optimal harvest window to maximize pennycress seed yield and oil content, characterize oil quality, and determine a range of cumulative growing degree days (CGDD) that correspond to pennycress physiological maturity. Pennycress was harvested at intervals throughout June at two locations as it matured and naturally senesced to determine indicators of plant physiological maturity. Parameters indicating physiological maturity (*e.g.*, maximum seed dry mass and oil content) were expressed in the same sequence regardless of location, and oil content was the final parameter to stabilize. Physiological maturity occurred between 2106°C d and 2153°C d CGDD, nearly two weeks before pennycress reached harvest maturity. Delaying pennycress harvest to harvest maturity at one location lead to a 68% loss in yield compared to the

average maximum yield of 1100 kg ha^{-1}. Ensuring maximum seed yield and oil content at pennycress harvest is imperative to successful production and contribution to farm economic viability. Therefore, it is important to harvest as soon after physiological maturity as possible due to potential yield loss.

2.2. INTRODUCTION

Within the omnipresent corn-soybean rotation in the Upper Midwest, there is a significant amount of time at the beginning and end of the growing season when land is underutilized. As demand for agricultural intensification rises, growers must leverage their land and resources in the most efficient manner possible (Heggenstaller et al., 2008; Heaton et al., 2013). One way to achieve this is through temporal intensification, a practice that integrates a secondary crop into the fallow shoulder seasons (mid- to late autumn and early to mid-spring) and can provide growers a suite of ecological and environmental services (Heaton et al., 2013). Despite an estimated 27 million ha in the Upper Midwest that can be utilized by a secondary crop without detracting from corn or soybean production, there is little adoption of this practice due to a lack of near-term profitability (Roesch-McNally et al., 2017; Sindelar et al., 2017; USDA NASS, 2017). Economic viability of a secondary crop is a major concern for growers. However, emerging opportunities are making this a more realistic prospect. One species of interest is field pennycress, an oil-producing member of the Brassicaceae family that is undergoing domestication (Sedbrook et al., 2014; Dorn et al., 2015) with potential for use as an industrial feedstock (Moser et al., 2009a; Fan et al., 2013). Moreover, field pennycress can be produced in a sustainably intensified manner that does not displace or reduce land availability for food production in the Upper Midwest (Johnson et al., 2015; Sindelar et al., 2017).

Brassicaceae species such as canola (*Brassicas napus* L.) and camelina (*Camelina sativa* [L.] Crantz) have traditionally been used as biofuel feedstocks due to their high yield and oil content (Moser et al., 2009b). Pennycress has gained attention as an alternative biodiesel feedstock due to its seed oil content and ability to survive the low winter temperatures of the Upper Midwest (Moser et al., 2009a; Moser, 2012; Sedbrook et al., 2014). Pennycress seed oil content ranges between 24 to 39% (wt/wt) and can yield up to 1200 L ha^{-1} of oil, making it favorable for biodiesel production (Moser et al., 2009a; Phippen and Phippen, 2013; Dose et al., 2017). This, in conjunction with its fluidity at low temperatures and high flash and fire points, makes it well suited for industrial applications (Moser et al., 2009b; Cermak et al., 2013).

The adoption of pennycress as a crop can improve farm economic viability while simultaneously providing ecosystem system services such as forage for pollinators and enhanced soil health and water quality. Recent research suggests that pennycress flowers bloom earlier than many other angiosperms and produce nectar and pollen for pollinators as early as the beginning of April in the Upper Midwest (Eberle et al., 2015). Pennycress is also able to improve soil and water quality by sequestering nutrients that would otherwise be susceptible to runoff from fallow fields during early spring snow melt and precipitation events and through tile flow. In comparison to soybean grown without a winter cover crop, including pennycress in the crop rotation significantly reduced the amount of extractable inorganic nitrogen in the soil profile to a depth of 60 cm (Johnson et al., 2017). This is due to the characteristic low growing rosette that develops prior to winter frost whose roots are able to take up soil nitrogen and whose vegetation is able to shield the soil from erosive forces (*e.g.*, wind, snowmelt, and rain) especially during early spring months (Fan et al., 2013; Johnson et al., 2015).

While pennycress shows great potential for economic viability as an oil-producing cover crop, there are significant yield-limiting barriers to production. Seed pod shatter at harvest is one of the largest sources of seed loss among Brassicaceae species, and pennycress is no exception (Pahkala and Sankari, 2001; Sedbrook et al., 2014). In studies on canola and camelina, seed loss can result from mechanical and environmental disturbances, and much of this loss is related to a decrease in plant moisture and advanced physiologic maturity (Vera et al., 2007; Sindelar et al., 2017; Sintim et al., 2016). In a study on canola, harvest losses at plant maturity equaled seven times the sown amount of seed and significantly contributed to the weed seed bank (Zhu et al., 2012). Seed loss due to shatter can result in persistent weed problems as seed remains viable for several years (Pahkala and Sankari, 2001; Sedbrook et al., 2014). Recent research has demonstrated that pennycress seed loss can exceed 60% of total seed harvested if proper harvest timing and management practices are not established (Carlson, 2018). The objectives of this study were to (1) identify the optimal harvest window that maximizes pennycress seed yield and oil content, (2) characterize changes in pennycress oil quality over multiple harvest intervals, and (3) determine a range of cumulative growing degree days corresponding to pennycress physiological maturity.

2.3. MATERIALS AND METHODS

Cultural practices

Field experiments were conducted during the 2016-2017 growing season at the USDA-ARS Swan Lake Research Farm near Morris, MN (45°41’12” N 95°48’12” W) and the University of Minnesota Rosemount Research and Outreach Center in Rosemount, MN (44°42’35” N 93°04’18” W). Soil at the Morris site was a Barnes silt loam (fine-loamy, mixed, superactive, frigid Calcic Hapludoll) and had a pH of 7.4. Total nitrogen content was 1.87 g kg^{-1} and organic matter content was 42 g kg^{-1} in the top 60 cm of soil. Soil at the Rosemount site was a well-drained Waukegan silt loam (fine silty over sandy, mixed mesic Typic Hapludoll) with a pH of 5.9. Total nitrogen content was 1.94 g kg^{-1} and organic matter content was 44 g kg^{-1} in the top 60 cm of soil. Monthly precipitation and mean air temperature for the study period can be found in Table 2-1. Weather parameters were recorded by weather stations at each site. The 30-year temperature and precipitation averages from 1981-2010 were obtained from sites in close proximity to the research sites (NOAA/NCEI, 2017). Averages from the West Central Research and Outreach Center, Morris, MN, were used for the Morris research site and is located approximately 18 km from the study location. Averages were available at the Rosemount Research and Outreach Center.

All plots were planted with ‘MN106’ field pennycress, a line selected from a natural pennycress population in Coates, MN, at a rate of 11.2 kg ha^{-1}. At Morris, pennycress was planted following a crop of spring wheat (*Triticum aestivum* L.). The seedbed was prepared by two passes of a no-till seed drill (approximately 3.8 cm deep soil disturbance). On the second pass, 1.1 kg a.i. ha^{-1} of trifluralin was incorporated for weed control. Pennycress seed was sown on 13 September 2016 at a depth of 0.6 cm with 19 cm row spacing using a plot drill (Great Plains Ag, Salina, KS) at Morris. At Rosemount, pennycress was planted following a third year alfalfa crop. Alfalfa was terminated with glyphosate [N-(phosphonomethyl) glycine] on 14 September 2016. The field was then prepared using a ripper followed by a field cultivator on 26 September 2016, and pennycress seed was broadcasted a day later using a Brillion seeder (Landoll Corp., Marysville, KS). Plot size was 1.5 m wide x 3.0 m long at both locations. Fertilizer was broadcast by hand in the spring soon after the soil had thawed at a rate of 79-34-34 kg ha^{-1} of N-P-K.

Treatments and plant analysis

Plots were arranged in a randomized complete block design with four replications. Treatment was harvest interval as pennycress naturally matured over the duration of the experiment. Pennycress was destructively hand harvested at eight intervals in Morris beginning on 1 June 2017 and at seven intervals in Rosemount beginning on 31 May 2017 (Tables 2-2 and 2-3, respectively). The first harvest interval at both locations was sampled when plants reached principal growth stage 7 and spanned to principal growth stage 9, when silicles reached full maturity, in accordance with the extended BBCH scale (Martinelli and Galasso, 2011). The sampling period was designed to encompass seed development from beginning fill through full maturity to target optimum harvest time to maximize seed yield and oil content.

All plots were hand harvested for biomass and seed yield by taking a 1-m^2 area from the center of the plot. Harvested plants were bagged and dried at 65°C for 48 h to constant mass before threshing and screen cleaning seed. This seed was tested for moisture and seed yield was adjusted to a moisture content of 80 g kg^{-1} (wt/wt). Crop harvest index was calculated as dry seed weight divided by dry weight of total aboveground biomass at harvest. Seed moisture at harvest was calculated by taking a second seed sample within the plot, outside the 1 m^2 harvest area. Seed moisture was determined by weighing seed samples after harvest and drying at 65°C to a constant mass. Cumulative growing degree days (°C d) were retroactively calculated for each harvest interval using daily maximum soil temperature (T_{max}), daily minimum soil temperature (T_{min}), and the established base temperature (T_b) of -2.5 °C (Eq. 1; Royo-Esnal et al., 2015).

$$CGDD = \sum \left(\frac{T_{max}+T_{min}}{2}\right) - T_b; \tag{1}$$

Seed oil content and fatty acid profile

Seed oil content was measured by pulsed nuclear magnetic resonance (NMR) (Bruker Minispec mq-10, Bruker, The Woodlands, TX, USA) using 6 g of seed from each replicated plot (n=4). Prior to NMR analysis, pennycress seed was dried at 130°C for 3 h and cooled in a desiccator for 30 min. The NMR instrument was calibrated with pure pennycress oil and values for oil content are reported as g kg^{-1} (dry wt oil/dry wt seed).

Fatty acid methyl ester (FAME) profiles of seed oil were measured by gas chromatography-mass spectroscopy (GC-MS) (Agilent 7890B GC and Agilent 5977B Networked MS). To profile the oil content, direct trans-esterification and conversion to fatty acid methyl esters (FAME) of ground seed (approximately 50 mg) was conducted using previously described methods (Griffiths et al., 2010; Lohman et al., 2014, 2015). GC-MS was performed using a 1 µL splitless injection into a 60 m × 0.25 mm Agilent DB-23 column as described in Lohman et al. (2014). The GC-MS was set with an injection temperature of 250°C using a Helium carrier gas and column at flow rate of 0.5 mL min^{-1} and a detector temperature of 150°C. The initial column temperature was 50°C and was increased to 175°C at a rate of 25°C min^{-1} followed by increments of 4°C min^{-1} to a final temperature of 230°C, which was held for 10 min before the run was terminated. The quantity of FAME for each sample was determined by integrating each FAME peak and compared against a standard curve (R^2>0.99) developed from a 37 FAME standard (Restek, Bellefonte, PA). Peak identification was based on retention time and NIST standard reference database using Agilent Mass Hunter Workstation software (ver. B07.00 Service Pack 2).

Statistical analysis

To determine the effect of harvest interval characterized by CGDD at each location on pennycress seed yield and oil content, locations were analyzed separately. Data analysis was performed using the MIXED procedure in SAS 9.4 (SAS Institute, 2013). Replication was considered as a random factor while CGDD was considered as a fixed factor. Treatment mean comparisons were made by using Tukey's HSD at $P < 0.05$.

Data for seed oil content and 1000-seed dry weight were plotted with respect to CGDD and fit using a logistic function where Y is mean oil content or 1000-seed dry weight, A is the maximum value of the response parameter, x is CGDD, b is the CGDD at which 50% of the response parameter occurred, and c is the slope of the regression line (Gesch and Johnson, 2013; Eq. 2).

$$Y = \frac{A}{1 + \left(\frac{x}{b}\right)^{c}}; \tag{2}$$

The CGDD at maximum response parameters were obtained by plotting the reference line, which denotes an average maximum of the Y parameter when no significant differences were observed. Seed moisture at physiological maturity was estimated the same way using 1000-seed dry weight plotted against seed moisture content and was determined based on average seed weight after it no longer changed with CGDD. A quadratic function was used to fit seed yield against CGDD at each location. The Akaike information criterion was used to aid in model selection by comparing quadratic and logistic models (Burnham and Anderson, 1998).

A multivariate analysis of variance (MANOVA) was used to determine whether harvest intervals, or CGDD, caused differences in palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), α-linolenic (C18:3), eicosenoic (C20:1), and erucic (C22:1) acid contents of pennycress using PROC GLM in SAS 9.3 (SAS Institute, 2013). The results of the MANOVA test were $p < 0.05$ using Pillai's Trace due to diffuse eigen values. Univariate test statistics of the harvest intervals were used to determine differences in FAME content using the MIXED procedure in SAS 9.4 where replication was considered a random effect and harvest date was a fixed effect (SAS Institute, 2013). Differences in means were assessed using the Tukey's HSD method with statistical significance at $P < 0.05$. A quadratic function was used to fit fatty acids versus CGDD (Berti and Johnson, 2008).

2.4. RESULTS

Environmental conditions

The 2016-2017 growing season was 2.1°C and 2.2°C warmer at Morris and Rosemount, respectively, compared to the 30-year averages for each location, and precipitation was normal at Morris, but departed from normal by an additional 113 mm at Rosemount (Table 2-1). Average temperatures at Rosemount were 1.4°C and 0.6°C higher than Morris during fall (September-November) and spring (March-June) periods of pennycress growth and development, respectively. Rosemount received 63 mm more precipitation in the fall and 165 mm more precipitation in the spring than Morris. Overall, conditions at Morris were much drier and slightly cooler than conditions at Rosemount over the duration of the growing season. Warm conditions and ample precipitation at Rosemount enabled pennycress to accumulate a comparable number of CGDD (1849 °C d) to Morris by the first harvest date despite the 13-day planting delay at Rosemount (Tables 2-2 and 2-3).

Measures of phenological development

At both locations, 1000-seed dry weight increased significantly with early harvest intervals, but values did not differ after 7 June (1985°C d CGDD) at Morris and 9 June (2075°C d CGDD) at Rosemount (Fig. 2-1; Tables 2-2 and 2-3). Following this maximization, values plateaued rapidly at Morris, but continued to increase at Rosemount for the duration of the trial (Fig. 2-1). Average maximum values of 1000-seed dry weight were 938 mg and 1348 mg and were modeled to have been reached by 2080°C d CGDD and 2212°C d CGDD at Morris and Rosemount, respectively. Meanwhile, seed moisture decreased significantly from 76% to 12% at Morris and 78% and 16% at Rosemount between the first and final harvest intervals (Tables 2-2 and 2-3). Both 1000-seed dry weight and seed moisture can be used as measurements of physiological maturity and, based on the modeled relationship between these parameters, physiological maturity may have occurred at seed moistures as high as 70% and 62% at Morris and Rosemount, respectively (Fig. 2-2).

There were no differences in above-ground biomass among harvest intervals at either location (data not shown), but seed yield statistically differed among harvest intervals at Morris (Fig. 2-3 and Table 2-2). This was reflected in the harvest index, the ratio between seed yield and above-ground biomass, which differed significantly between harvest intervals at Morris, but not at Rosemount (Tables 2-2 and 2-3). Seed yield at Morris increased significantly and peaked between 9 June (2034°C d CGDD) and 19 June (2261°C d CGDD) before decreasing significantly. Maximum modeled seed yield at Morris was 1187 kg ha^{-1} and was estimated to occur at 2144°C d CGDD when pennycress silicles were between 10% and 70% mature (Fig. 2-3 and Table 2-2). Seed yield at Rosemount increased throughout the trial, however, the yields obtained at each harvest interval were not statistically different from each other. Maximum modeled seed yield at Rosemount was estimated to be 969 kg ha^{-1} at 2235°C d CGDD (Fig. 2-3 and Table 2-3).

Development of seed oil content followed a similar trend at both locations and increased significantly until 12 June, which corresponded to 2106°C d and 2153°C d CGDD at Morris and Rosemount, respectively (Fig. 2-4; Tables 2-2 and 2-3). Oil content did not significantly differ between locations and ranged between 243 g kg^{-1} and 333 g kg^{-1} over the last five harvest intervals as oil levels stabilized. Oil content stabilized around 330 g kg^{-1} at Morris and 310 g kg^{-1} at Rosemount (Fig. 2-4). Seed oil content was modeled with respect to CGDD and was estimated to maximize by 2201°C d CGDD at Morris and 2272°C d CGDD at Rosemount, which was 57°C d and 37°C d later than when seed yield was modeled to maximize at Morris and Rosemount, respectively (Figs. 2-3 and 2-4).

Seed maturation and quality

Seed carbon increased with harvest interval as seed matured at both locations (Tables 2-2 and 2-3). At Morris, seed carbon increased from the beginning of the trial until 12 June (2106°C d CGDD) by 16%, but, following this, did not change significantly (Table 2-2). Similarly at Rosemount, seed carbon increased by 23% until 12 June (2153°C d CGDD) and did not change with following harvest intervals (Table 2-3). Average maximum seed carbon was 577 g kg^{-1} and 562 g kg^{-1} at Morris and Rosemount, respectively (Tables 2-2 and 2-3). Crude protein followed a similar trend to seed carbon and increased at both locations until 15 June (2175°C d CGDD at Morris and 2229°C d CGDD at Rosemount) (Tables 2-2 and 2-3). Maximum crude protein averaged 237 g kg^{-1} and 238 g kg^{-1} at Morris and Rosemount, respectively.

Overall, development of major fatty acids followed similar trends at both locations with comparable final levels (Figs. 2-5 and 2-6). Between the first and final harvest intervals there was an increase in oleic, eicosenoic, and erucic acids and a decrease in linoleic and linolenic acids. The final composition of major fatty acids was reached by 7 June (1985°C d CGDD) at Morris, but did not stabilize at Rosemount until 12 June (2153°C d CGDD) (Figs. 2-5 and 2-6). Erucic acid increased with harvest intervals as linoleic and linolenic acid contents decreased (Figs. 2-5 and 2-6). At both locations, final erucic, linoleic, and linolenic acid contents stabilized around 40%, 20%, and 10%, respectively (Figs. 2-5 and 2-6). Both oleic and eicosenoic acids reached maximum values early in the trial and remained stable thereafter (Figs. 2-5 and 2-6). Oleic acid reached its maximum value of about 10% by the second harvest interval at both locations, 7 June (1985°C d CGDD) at Morris and 5 June (1971°C d CGDD) at Rosemount. Similarly, eicosenoic acid reached its maximum value of approximately 8% by the second harvest interval at Morris, 7 June (1985°C d CGDD), and by the third harvest interval at Rosemount, 9 June (2075°C d CGDD).

2.5. DISCUSSION

Pennycress phenological development

Prior studies of pennycress have not attempted to characterize growth stage at points other than at harvest maturity, which occurs when 90% of pennycress silicles are mature. The extended BBCH scale for camelina principally uses seed color as a proxy for maturity (Martinelli and Galasso, 2011), and while pennycress seed matures from green to brown seed, this may not be the best way to determine growth stage. One study suggested qualitatively measuring plant maturity by pod color on a four-point scale ranging from green to fully dry silicles, however, this designation does not account for the continuum of maturation throughout the ripening process (Claver et al., 2017). In the future, growth stage of pennycress should be interpreted using an adapted version of the extended BBCH scale that is based on silicle, rather than seed, ripeness with harvest maturity occurring when 90% of pods have dried and yellowed (Altendorf, 2017). Previous results for camelina and canola similarly suggest that harvest maturity occurs when 90% of seeds on a plant are mature (Elias and Copeland, 2001; Masella et al., 2014; Walia et al., 2018).

Harvest attributes as an indicator of physiological maturity

Thousand-seed dry weight was used to estimate pennycress physiological maturity, which occurred when the seed reached maximum dry weight (Elias and Copeland, 2001). Average maximum 1000-seed dry weight was comparable to reported values ranging between 800-1500 mg depending on genetic line and study conditions (Phippen and Phippen, 2013; Zanetti et al., 2013; Sedbrook et al., 2014). Maximization of 1000-seed dry weight indicates that physiological maturity occurred earlier than harvest maturity by 274°C d at Morris and 164°C d at Rosemount. A study by Vera et al. (2007) determined that physiological maturity in canola occurred around 40% seed moisture meaning that, if pennycress behaves similarly, physiological maturity occurred between 15 June (2175°C d) and 19 June (2261°C d) at Morris and between 15 June (2229°C d) and 19 June (2327°C d) at Rosemount. While attainment of physiological maturity when seed moisture is too high for mechanical harvest, cultural practices, such as the use of a desiccant or swathing, may be utilized to reduce the time between crop physiological and harvest maturities (Berti and Johnson, 2008; Sintim et al., 2016). The role of harvest aids, such as swathing and desiccants, in cropping systems will be the focus of future studies to address the issue of early harvest to reduce yield loss.

Average pennycress seed yield for the Upper Midwest ranges between 500 kg ha^{-1} and 1100 kg ha^{-1} (Johnson et al., 2015, 2017; Dose et al., 2017), which encompasses the values at Rosemount in the present study. However, the modeled maximum seed yield at Morris exceeded the averages previously reported for this region and delaying pennycress harvest to harvest maturity lead to a 68% loss in yield compared to the maximum. Rosemount did not exhibit loss of seed yield associated with low seed moisture, presumably due to silicle shattering. The Morris site had one additional sampling date in comparison to Rosemount, and lower moisture content and advanced phenological growth stage at the final two Morris sampling dates may have influenced the sharp decrease in yield at the 23 and 27 June harvest intervals. Yield loss due to silicle shatter is not uncommon when pennycress is harvested at harvest maturity, and similar to the Morris site in this study, over 60% loss of pennycress seed due to shatter has been reported when harvest occurs at harvest maturity (Carlson 2018). Studies of canola have estimated shatter loss between 10% and 25% when plants are harvested at harvest maturity, and current best management practices indicate that swathing canola when 15% to 25% of seed is mature can reduce yield loss due to shatter (Price et al., 1996; Gugel and Falk, 2006; Kandel and Hanson, 2013). Based on the maximization of seed yield in the present study, pennycress physiological maturity occurred 264°C d and 331°C d earlier than harvest maturity at Morris and Rosemount, respectively.

Maximum oil content was 330 g kg^{-1} at Morris and 310 g kg^{-1} at Rosemount, which was similar to previously reported results (Moser et al., 2009a; Dose et al., 2017). While seed yield reached maximum values at Morris before oil content stabilized, the maximum oil content occurred within the range of harvest intervals encompassing maximum seed yield, which indicates that late stabilization of oil content with respect to other parameters of physiological maturity may not interfere with harvest for maximum seed yield. Given that oil content is vital to achieving maximum oil yield and was the last major parameter to stabilize, an important recommendation is to delay pennycress harvest until seed oil content is fully developed. However, it is also important to consider seed yield trade-offs of delaying harvest. An earlier harvest with a greater seed yield has the potential to compensate for slightly lower seed oil content when compared to a delayed harvest to stabilize oil content, which may increase the risk of loss due to silicle shatter. Furthermore, the differences in CGDD between the maximization of seed yield and oil content equates to a one-day difference in harvest, so it is more important to target the correct window for harvest rather than a specific day.

Seed fatty acid profile

Despite differences in accession origin when compared to previously characterized wild-type pennycress lines, levels of the various fatty acid components of pennycress oil in this experiment were consistent with prior studies. Erucic acid was the most abundant oil component at both locations and comprised close to 35% of the total oil content. This erucic acid value is consistent with prior research, which reported values between 29 and 36% (Isbell, 2009; Moser et al., 2009b; Cermak et al., 2013; Phippen and Phippen, 2013). Oleic acid is the precursor of linoleic, linolenic, eicosenoic, and erucic acids (Sedbrook et al., 2014), and the development of these fatty acids may account for the low levels of oleic acid in pennycress oil. The levels of other major fatty acids in the current study were consistent with prior pennycress oil characterization studies (Moser et al., 2009a; Cermak et al., 2013). At both locations, accumulation of major fatty acids occurred prior to all other measures of physiological maturity except seed yield at Rosemount.

2.6. CONCLUSIONS

Field pennycress has great potential as an oilseed cover crop in the Upper Midwest for use in biofuels due to its winter growth habit and hardiness, seed yield potential, and consistently high oil content. Pennycress performed slightly differently between locations in Minnesota in terms of accumulation of CGDD for each parameter of physiological maturity (e.g., 1000-seed dry weight, seed moisture, seed yield, and oil content), however, the relative timing of these parameters followed the same sequence regardless of location. While these parameters varied in their timing of maximization, an important consideration is how pennycress will be applied as a crop in the future. For growers, achieving maximum seed yield and oil content and quality are the most important factors contributing to successful production. In terms of these variables, pennycress reached physiological maturity before 12 June (2106°C d at Morris and

2153°C d at Rosemount), which indicates that it is ready for harvest despite high seed moisture values.

To ensure that pennycress is a viable crop for mechanical harvest, seed moisture values must be decreased prior to harvest and near-term research will focus on the use of swathing and desiccants to achieve earlier harvest dates. In the long-term, however, breeding for non-shattering genotypes will be important. Delaying harvest to harvest maturity and beyond can have dramatic consequences due to the increased potential of yield loss due to pod shatter. Harvest of pennycress as close to physiological maturity as possible will be important for the greatest profitability from the crop. Early harvest of pennycress will allow for it to be double cropped with short season annual crops, further promoting farm economic viability.

2.7. TABLES AND FIGURES

Table 2-1. Mean monthly air temperatures and accumulated precipitation during the study period of 2016-2017 and departure from 30-yr average at each location.

	Morris, MN				Rosemount, MN			
	†Mean air temperature °C		Cumulative precipitation mm		Mean air temperature °C		Cumulative precipitation mm	
Month	2016-2017	‡Departure from 30-yr average	2016-2017	Departure from 30-year average	2016-2017	Departure from 30-year average	2016-2017	Departure from 30-year average
September	16.7	-1.7	42.9	-30.8	18.1	1.9	132.8	40.6
October	9.6	2.1	87.1	22.8	11.1	2.2	62.2	-10.4
November	4.8	-6.1	42.2	14.8	6.2	6.1	45.2	-8.1
December	-9.2	0.2	32.5	14.2	-7.1	1.1	24.1	-6.9
January	-9.1	3.1	13.2	-5.6	-7.6	3.1	51.8	-25.4
February	-2.8	6.6	11.4	-6.6	-1.9	5.8	16.0	-7.1
March	-0.9	1.4	11.7	-24.6	-0.8	0.3	16.0	-42.4
April	7.6	1.0	65.0	6.3	9.0	1.1	115.6	41.4
May	13.5	0.0	92.5	20.6	13.5	-0.8	182.4	79.8
June	19.8	-0.7	101.1	-0.8	20.5	0.9	91.4	-28.5
Average/Total	5.0		489.3		6.2		766.9	

†Mean air temperature and accumulated precipitation were recorded from weather stations at the Swan Lake Research Farm, Morris, MN and the Rosemount Research and Outreach Center, Rosemount, MN.

‡30-year averages for both locations were calculated averages from 1981-2010 from NOAA/NCEI (2017).

Table 2-2. Phenological growth stage, seed moisture at harvest, harvest index, seed carbon, and crude protein content of pennycress as influenced by harvest date at Morris, MN, 2017.

Harvest date	CGDD (˚C d)	Phenological growth stage†	Seed moisture at harvest (%)	Harvest index	Seed carbon (g kg^{-1})	Crude protein (g kg^{-1})
June 1	1849	77.5 g	76.2 a	0.08 d	470 d	167 e
June 7	1985	80.0 f	73.3 ab	0.22 bc	526 c	203 d
June 9	2034	81.0 e	67.5 b	0.31 ab	557 b	216 c
June 12	2106	86.6 d	66.3 b	0.24 a	575 a	227 bc
June 15	2175	86.6 d	45.9 c	0.34 a	577 a	233 ab
June 19	2261	88.5 c	36.4 d	0.33 a	581 a	238 ab
June 23	2348	89.0 b	33.4 d	0.27 ab	578 a	239 a
June 27	2420	90.0 a	12.0 e	0.16 cd	576 a	236 ab
Average	-	84.9	51.0	0.26	555	220
P>F‡		<.0001	<.0001	<.0001	<.0001	<.0001

†According to the extended BBCH scale (Martinelli and Galasso, 2011).

‡Significance of F test. Values within columns followed by the same letter are not significantly different using Tukey's HSD at the $p < 0.05$ level. Values represent means ($n = 4$).

Table 2-3. Phenological growth stage, seed moisture at harvest, harvest index, seed carbon, and crude protein content of pennycress as influenced by harvest date at Rosemount, MN, 2017.

Harvest date	CGDD (˚C d)	Phenological growth stage‡	Seed moisture at harvest (%)	Harvest index	Seed carbon (g kg^{-1})	Crude protein (g kg^{-1})
May 31	1849	77.0 g	77.6 a	0.12	476 d	184 d
June 5	1971	80.1 f	73.0 a	0.24	506 c	206 c
June 9	2075	80.2 e	68.7 ab	0.27	537 b	221 bc
June 12	2153	84.7 d	66.2 ab	0.27	551 ab	230 ab
June 15	2229	85.7 c	54.4 b	0.28	559 ab	238 a
June 19	2327	88.0 b	26.1 c	0.24	568 a	240 a
June 21	2376	88.8 a	15.9 c	0.26	569 a	242 a
Average	-	83.5	55.8	0.25	538	223
P>F†		<.0001	<.0001	0.21	<.0001	<.0001

†According to the extended BBCH scale (Martinelli and Galasso, 2011).

‡Significance of F test. Values within columns followed by the same letter are not significantly different using Tukey's HSD at the $p < 0.05$ level. When no letters are shown, this indicates there was no significant effect. Values represent means ($n = 4$).

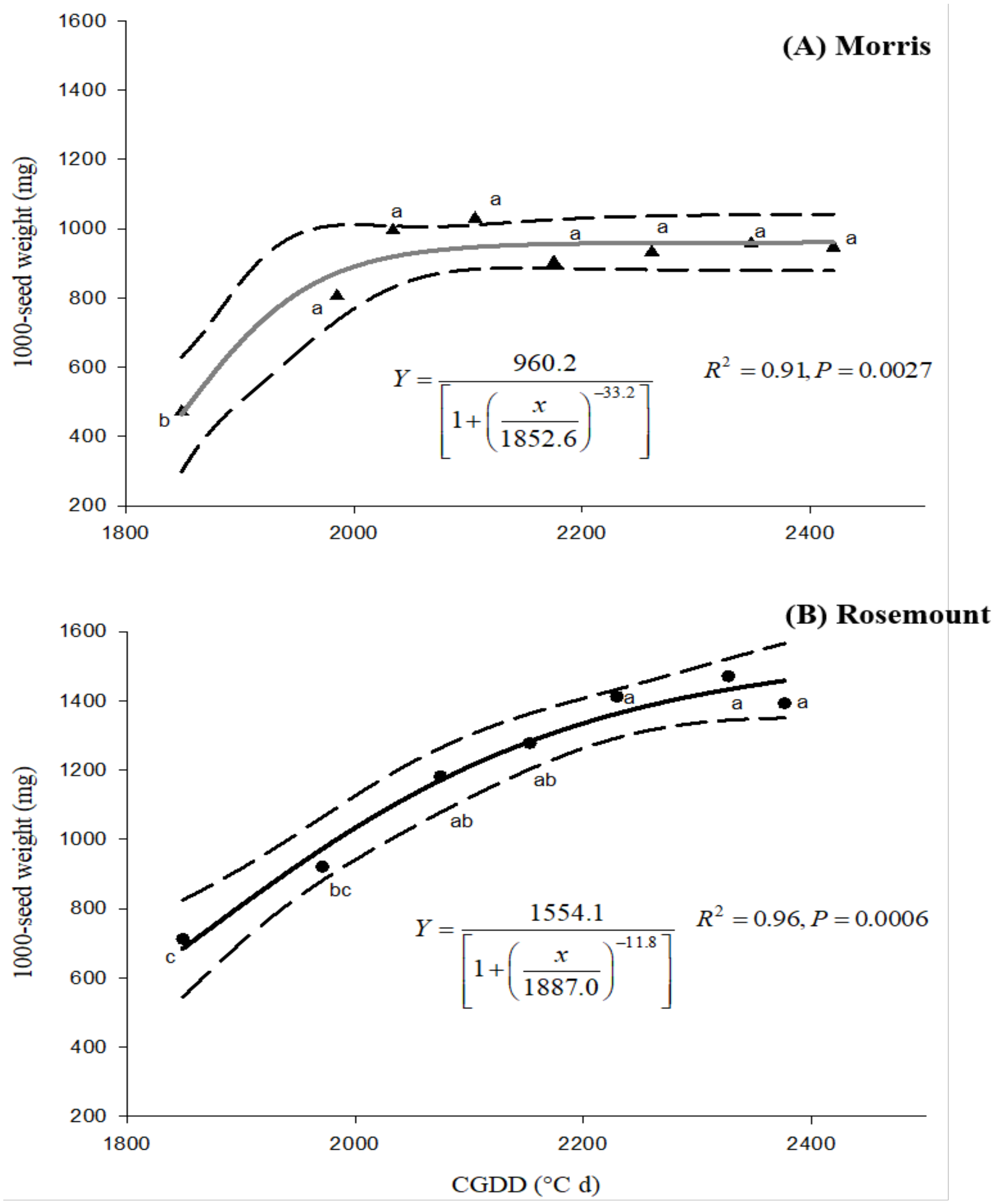

Figure 2-1. Relationship of 1000-seed weight as a function of CGDD at (A) Morris and (B) Rosemount, MN, 2017. Individual values are means, $n = 4$. Values followed by the same letter at each location are not significantly different using Tukey's HSD at the $P < 0.05$ level. The dashed lines mark the 95% confidence interval.

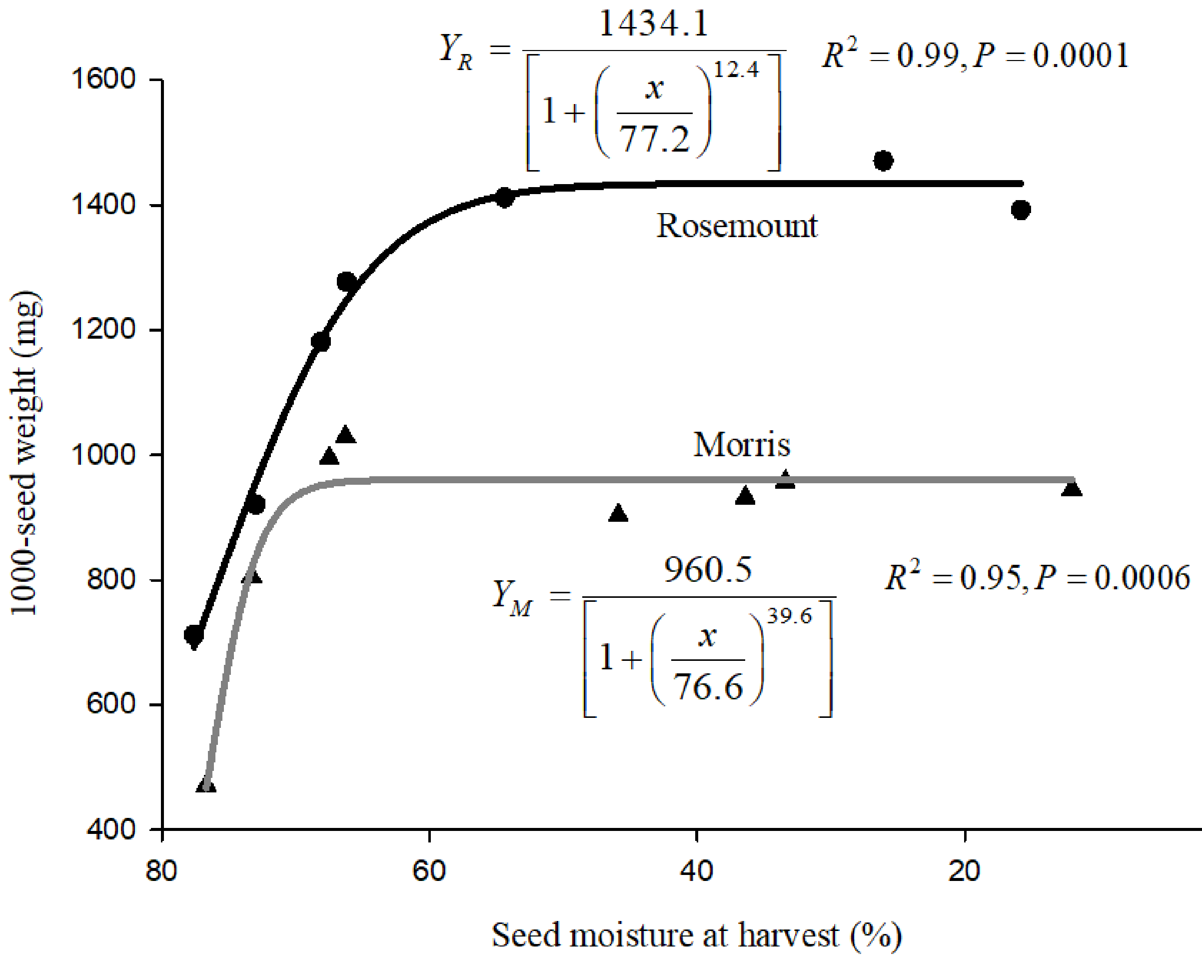

Figure 2-2. Relationship of 1000-seed weight as a function of seed moisture at harvest at Morris and Rosemount, MN, 2017. Individual values are means, *n* = 4. Triangular symbols along with grey fit line represents Morris, while circular symbols along with black fit line represents Rosemount. Y_M and Y_R in equations represent response parameters, i.e., 1000-seed weights at Morris and Rosemount, respectively.

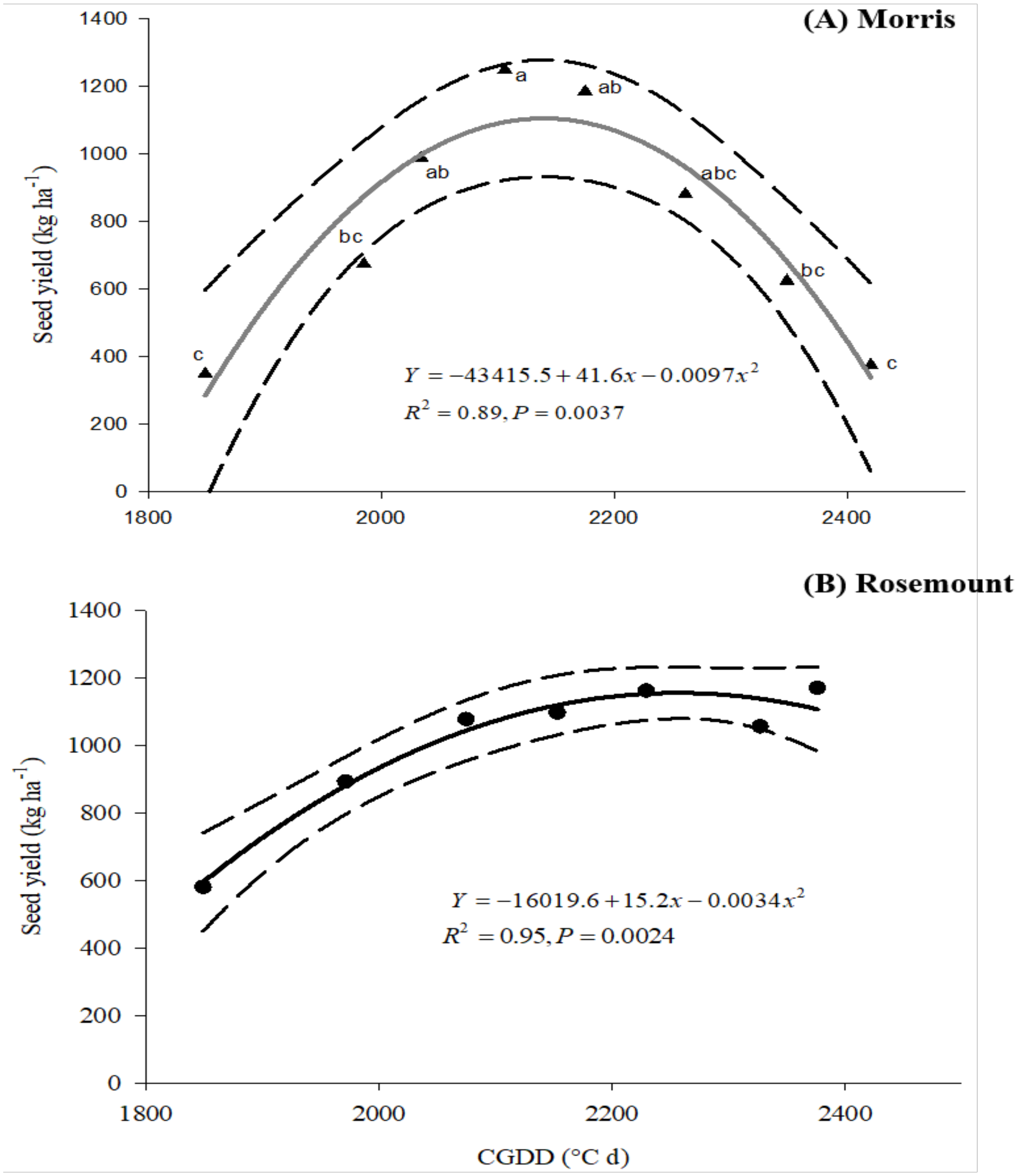

Figure 2-3. Relationship of seed yield as a function of CGDD at (A) Morris and (B) Rosemount, MN, 2017. Individual values are means, $n = 4$. Values followed by the same letter at each location are not significantly different using Tukey's HSD at the $P < 0.05$ level. When no letters are shown, this indicates there was no significant effect. The dashed lines mark the 95% confidence interval.

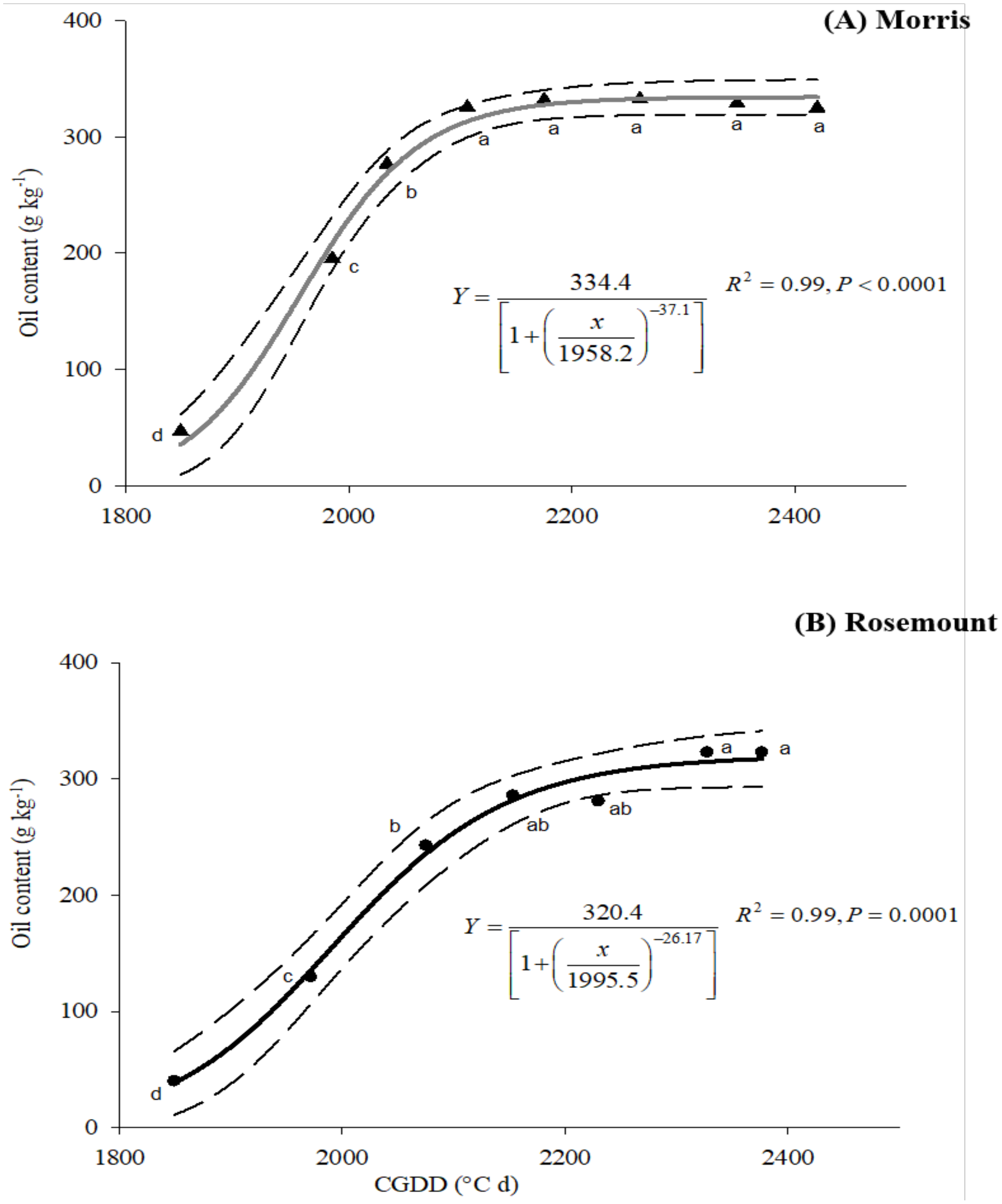

Figure 2-4. Relationship of seed oil content as a function of CGDD at (A) Morris and (B) Rosemount, MN, 2017. Individual values are means, $n = 4$. Values followed by the same letter at each location are not significantly different using Tukey's HSD at the $P < 0.05$ level. The dashed lines mark the 95% confidence interval.

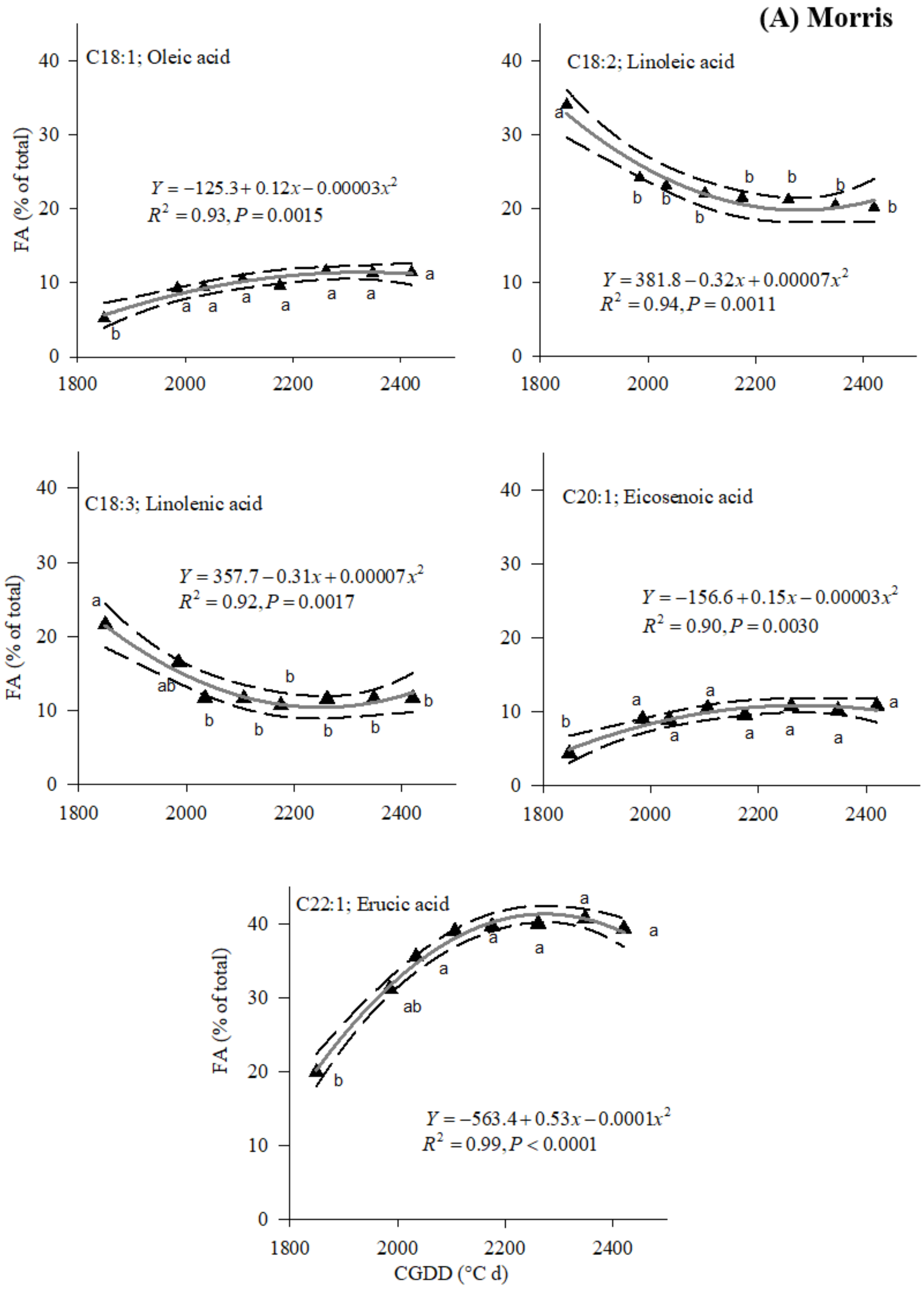

Figure 2-5. Relationship of major fatty acids (% of total oil) in the seed oil as a function of CGDD at Morris, MN, 2017. Values followed by the same letter are not significantly different using Tukey's HSD at the $P < 0.05$ level. Values represent means ($n = 4$). The dashed lines mark the 95% confidence interval for each fatty acid.

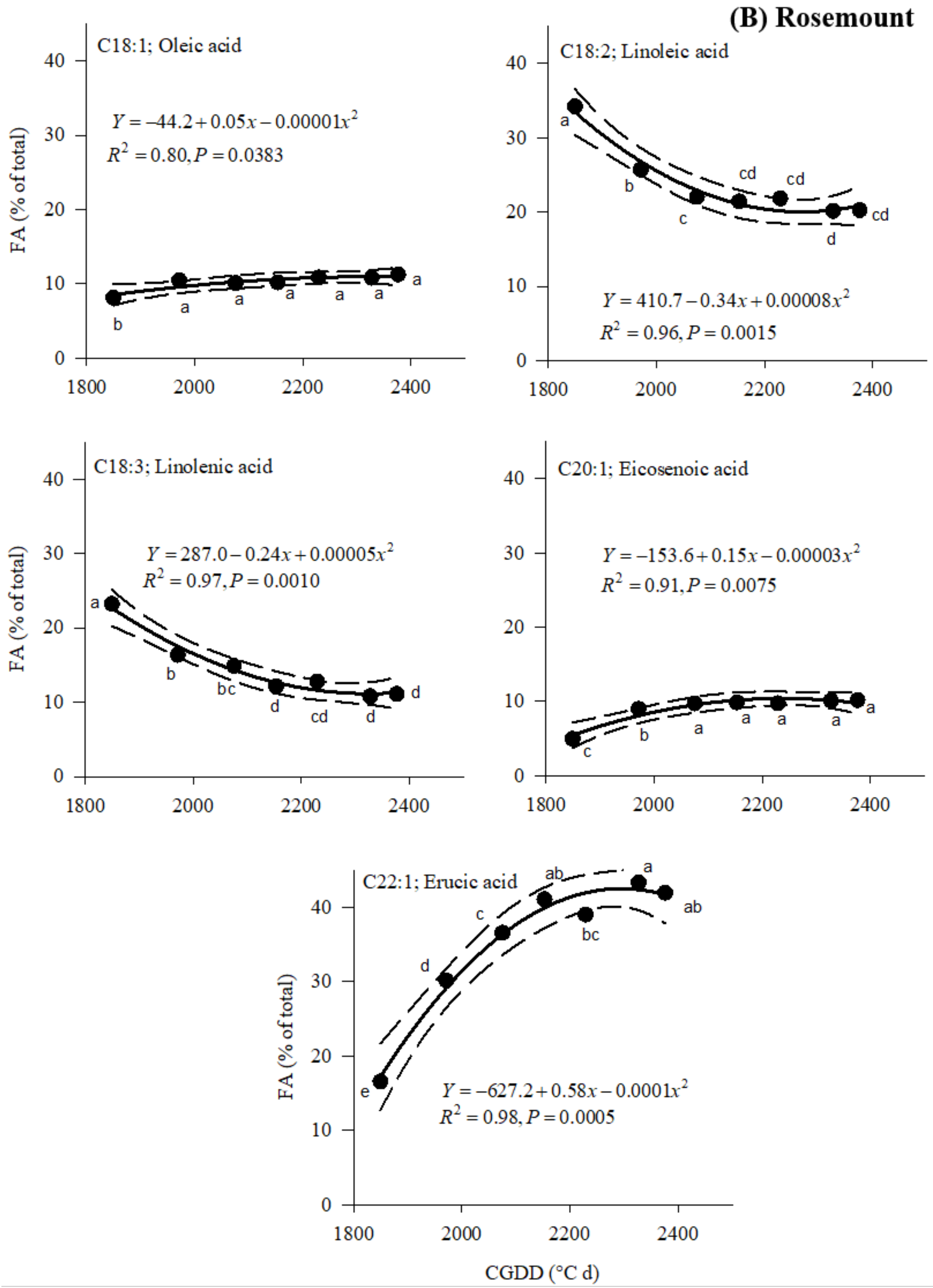

Figure 2-6. Relationship of major fatty acids (% of total oil) in the seed oil as a function of CGDD at Rosemount, MN, 2017. Values followed by the same letter are not significantly different using Tukey's HSD at the $P < 0.05$ level. Values represent means ($n = 4$). The dashed lines mark the 95% confidence interval for each fatty acid.

3. PENNYCRESS-SOYBEAN DOUBLE CROP OPTIMIZATION AS DETERMINED BY YIELD AND ECONOMIC TRADE-OFFS

3.1. SUMMARY

As growers look to maximize use of the short growing season in the Upper Midwest, economically-viable alternative cropping systems within the corn (*Zea mays* L.)-soybean [*Glycine max* (L.) Merr.] rotation are of special interest. Pennycress (*Thlaspi arvense* L.) is a winter annual oilseed crop that can expand the economic opportunities of growers without jeopardizing summer annual production. Field experiments were conducted to (i) maximize annual oilseed production by optimizing timing of pennycress harvest and soybean planting, (ii) assess pennycress yield loss due to silicle shatter, and (iii) evaluate economic trade-offs of integrating pennycress into a summer annual rotation. Field experiments were conducted over four site-years in Minnesota where fall-planted pennycress was desiccated and harvested at intervals throughout June prior to soybean planting. Mono-cropped soybean yielded more prolifically than double-cropped soybean, however, when pennycress and soybean were combined, the aggregated double crop yield produced 12% more oilseed than the soybean monocrop alone in one site-year. Pennycress seed shatter appeared to inhibit soybean germination when pennycress was $\geq$ 70% maturity, but did not have a significant effect on pennycress yield. Economically, the double crop system performed poorly in comparison to the mono-cropped scenarios and had a negative profit of \$638.42 and \$239.50 ha^{-1} for each experimental design. Upon optimization of the double crop system, pennycress may be an attractive option to provide both a winter cover crop and an additional cash crop in the corn-soybean

rotation, but these experiments provide evidence that further optimization and research is required before it is economically viable.

3.2. INTRODUCTION

Agricultural land in the Upper Midwest of the United States is dominated by the two-year summer annual rotation of corn and soybean. This cropping system does not maximize the use of annual growing degree days and has normalized an environmentally-problematic fallow off-season between late fall and early- to mid-spring (Strock et al., 2004; Randall and Vetsch, 2005; Heggenstaller et al., 2008). One method to increase growing degree day utilization and decrease this fallow period is through sustainable intensification, a practice where more than one crop is harvested off the same parcel of land in a given growing season (Heaton et al., 2013). This is typically accomplished by incorporating a winter annual into the summer annual rotation through double- or relay-cropping (Heaton et al., 2013). However, in regions like the Upper Midwest that are characterized by a short growing season, a limited number of seasonal growing degree days, and frigid winter temperatures, integrating an additional crop can be challenging (Sindelar et al., 2017). While there are a number of cover crops, such as winter rye (*Secale cereale* L.) and red clover (*Trifolium pratense* L.), that physiologically fit these conditions, another important requirement is economic marketability (Roesch-McNally et al., 2017). While traditional cover crops provide ecologic benefits, they lack near-term profitability on farms that do not have the ability to graze ruminant livestock (Roesch-McNally et al., 2017).

There is great need for crops that can provide both ecologic and economic benefits to growers, which has generated interest in winter-hardy, oil-rich crops such as pennycress (Heaton et al., 2013; Sindelar et al., 2017). Though native to Eurasia, accessions of naturalized pennycress have been found throughout temperate regions worldwide, including the Upper Midwest, demonstrating adaptability to the regional climate (Warwick et al., 2002). As a winter annual, pennycress is planted in early fall, produces a basal rosette before winter (though seed dormancy issues can lead to spring, rather than fall, germination), bolts following spring thaw, and is harvested for seed in late spring (Warwick et al., 2002). This winter growth habit allows pennycress to maximize underutilized growing degree days and provide ground cover during the fallow shoulder season of the corn-soybean rotation when soil would otherwise be vulnerable to erosive forces (*i.e.* heavy wind, snow melt, precipitation). In addition to protective cover from erosion, pennycress is also able to scavenge available nutrients from the soil, which is of particular importance in the corn-soybean rotation where 73-77% of nitrate loss occurs between April and June (Randall and Vetsch, 2005). Johnson et al. (2017) demonstrated that soil nitrogen levels were significantly reduced when soybean was double cropped with pennycress in comparison to a sole soybean crop. The double cropped system decreased soil nitrogen by 18-19% in the fall after oilseed planting and by 53-72% in the spring following oilseed harvest when compared to the soybean monocrop (Johnson et al., 2017). Despite these benefits, pennycress may be potentially problematic for growers as it is susceptible to seed loss due to silicle shatter, a common issue in Brassicaceae species (Pahkala and Sankari, 2001; Sedbrook et al., 2014).

Delaying pennycress harvest past physiological maturity, when seed reaches its maximum dry matter, corresponded to 76% seed loss in comparison to plants harvested at physiological maturity (Cubins et al., unpublished). This introduces an additional time-sensitive component in integrating pennycress into a cropping rotation.

Despite these challenges, a double-cropped system may be economically attractive to growers in comparison to growing solely a summer annual due the potential increase in total annual seed yield and diversification of markets. Camelina (*Camelina sativa* L.), a closely related Brassicaceae species to pennycress, has been successfully double cropped with soybean in west central Minnesota without reducing soybean quality, and aggregate camelina and soybean yield was greater than that of the soybean monocrop (Gesch et al., 2014). However, to accommodate late planting and ensure maturation before frost, a shorter season soybean (MG 00) was used when compared to the full season control (MG 1.3), which was associated with a decrease in soybean yield (Gesch et al., 2014). This has been reflected in recent pennycress research conducted in southern Minnesota where soybean maturity group was adjusted from MG II to MG I to account for the later soybean planting date (Johnson et al., 2017). As expected, annual oilseed yield (*i.e.*, combined pennycress and soybean) significantly increased by 48-102% in a double-cropped system even in years where soybean yield decreased due to early frost or the use of a shorter season soybean (Johnson et al., 2015, 2017). Other studies conducted at lower latitudes (*e.g.*, Illinois, Germany) have shown that a pennycress double crop does not have an impact on soybean yield or maturation (Phippen and Phippen, 2012; Groeneveld and Klein, 2014). However, to decrease the risk of an immature soybean crop, it is likely that double-cropped pennycress in the Upper Midwest will require the use of a lower soybean maturity group. Under optimal production conditions, it is suggested that pennycress can produce between 600-1200 L of oil ha^{-1} (Moser et al., 2009a) as compared to 450 L of oil ha^{-1} for soybean (Marek et al., 2008) making it a favorable oil producer. Markets for pennycress oil are in development because of its potential use as an industrial biofuel feedstock (Moser et al., 2009a).

There are agronomic challenges and potential risks incurred when double cropping pennycress with soybean. Harvesting pennycress too late may increase silicle fragility and decrease seed yield due to disturbance, and planting soybean too late or not decreasing the maturity group may also decrease its yield potential. The objectives of this study were to address the known major challenges of the pennycress-soybean double-crop by (i) maximizing annual oilseed production by optimizing timing of pennycress harvest and soybean planting date, (ii) assessing pennycress yield loss due to silicle shatter, and (iii) evaluating economic trade-offs of integrating pennycress into a summer annual rotation.

3.3. MATERIALS AND METHODS

Field experiments were conducted at two sites over the 2016-2017 and 2017-2018 growing seasons. In both years, experiments were conducted at the USDA-ARS Swan Lake Research Farm near Morris, Minnesota (45°41’12” N, 95°48’12” W) and the University of Minnesota Rosemount Research and Outreach Center in Rosemount, Minnesota (44°43’24” N, 93°06’23” W). Two distinctly different experiments were conducted at each research site. Both sites looked at the effect of pennycress desiccation on pennycress, soybean, and aggregate seed and oil yield. The Morris site, hereby referred to as Experiment 1, assessed soybean yield with respect to planting date. The Rosemount site, hereby referred to as Experiment 2, assessed soybean yield with respect to maturity group.

Experiment 1. Pennycress, soybean, and aggregate oilseed yield as influenced by pennycress desiccation and soybean planting date

Soils at the Morris site over the 2016-2017 growing season were a well-drained Hokans-Svea complex (fine-loamy, mixed, superactive, frigid Calcic Hapludolls) with a pH of 7.4. Total nitrogen content (inorganic nitrate and ammonium) was 1.87 g kg^{-1} and organic matter content was 42 g kg^{-1} in the top 15 cm of soil. Soil at the Morris site over the 2017-2018 growing season was a moderately well-drained Balaton-Hamerly complex (fine-loamy, mixed, superactive, frigid Aquic Calciudolls). The pH was 7.1, total nitrogen content was 1.85 g kg^{-1}, and organic matter content was 53 g kg^{-1} in the top 15 cm of soil. Monthly precipitation and mean air temperature for the study period can be found in Table 3-1. Weather parameters were recorded by a weather station located at the Swan Lake Research Farm. The 30-year temperature and precipitation averages from 1981-2010 were obtained from the West Central Research and Outreach Center, located approximately 18 km from the study location (NOAA/NCEI 2017).

In both experimental years, all plots were planted with ‘MN106’ field pennycress, a line selected from a natural pennycress population in Coates, MN, at a rate of 11.2 kg ha^{-1} following a crop of spring wheat (*Triticum aestivum* L.). The seedbed was prepared by two passes of a no-till seed drill and 1.1 kg a.i. ha^{-1} of trifluralin was incorporated into the soil for weed control on the second pass. Pennycress seed was sown on 13 September and on 16 September in 2016 and 2017, respectively, using a plot drill with 19 cm row spacing. Plot size was 3.0 m wide x 7.6 m. Plots were fertilized with 79-34-34 kg ha^{-1} N-P-K on 30 March 2017 and 11 May 2018 following spring snow melt with a 50' broadcast spreader. A summary of pennycress field management practices can be found in Table 3-2.

Plots were arranged in a randomized complete block design with four replications. Treatment was pennycress desiccation date and controls were mono-cropped soybean and naturally senesced pennycress (Table 3-3). Prior to desiccant application, pennycress plots were assessed for phenology and measured for plant height (data not shown). Defol 5 ($NaClO_3$) (Drexel Chemical, Memphis, TN) was applied to pennycress at a rate of 11.3 L a.i. ha^{-1} over four time points, each representing a differing stage of pennycress maturity. Desiccation began on 5 June in 2017 and 15 June in 2018. Following desiccation and when pennycress had reached harvest maturity (Martinelli and Galasso, 2011), pennycress was harvested using a plot combine. Combined seed was then bagged and dried at 65°C for 48 h to constant mass. This seed was tested for moisture and seed yield was adjusted to a moisture content of 80 g kg^{-1} (wt/wt).

All soybeans (ASGROW AG1135, MG 1.1) were planted using a four-row planter at a rate of 420,078 seeds ha^{-1}. Control soybeans were no-till drilled into plots containing pennycress that had been terminated with glyphosate [N-(phosphonomethyl) glycine] following spring thaw. Control soybeans were planted on 11 May in 2017 and on 22 May in 2018. All other soybeans were no-till drilled following pennycress senescence and harvest (Table 3-3). Control soybeans were machine harvested with a plot combine on 12 October and 22 October in 2017 and 2018, respectively, and double cropped soybeans were harvested on 25 October in 2017 and on 31 October in 2018. A summary of soybean field management practices can be found in Table 3-2. Following soybean harvest, seed was bagged and dried at 65°C for 48 h to constant mass. This seed was tested for moisture and seed yield was adjusted to a moisture content of 130 g kg^{-1} (wt/wt).

Experiment 2. Pennycress, soybean, and aggregate oilseed yield as influenced by pennycress desiccation and soybean maturity group

Rosemount soil type was consistent over both years of the study and was a Waukegan silt loam (fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludolls). In 2017, the pH was 5.9, total nitrogen content was 1.94 g kg^{-1}, and organic matter content was 44 g kg^{-1}. In 2018, soil had a pH of 6.5, total nitrogen content of 1.99 g kg^{-1}, and organic matter content of 45 g kg^{-1}. Monthly precipitation and mean air temperature for the study period can be found in Table 3-4. Weather parameters were recorded by weathers stations at the Rosemount Research and Outreach Center. The 30-year temperature and precipitation averages from 1981-2010 were also obtained from the Rosemount Research and Outreach Center (NOAA/NCEI 2017).

In both years, plots were 3.0 m x 7.6 m, planted with ‘MN106’ field pennycress, and arranged in a randomized complete block design with four replications. Prior to the 2017 growing season, pennycress was planted following third year alfalfa (*Medicago sativa* L.). Alfalfa was terminated with glyphosate on 14 September 2016 and the field was then prepared using a ripper followed by a field cultivator on 26 September 2016. Pennycress seed was broadcast seeded using a Brillion seeder (Landoll Corp., Marysville, KS) on 27 September 2016 at a rate of 11.2 kg ha^{-1}. Following spring thaw, 79-34-34 kg ha^{-1} N-P-K was broadcast by hand on 5 May 2017. Prior to the 2018 growing season, the field was prepared using a chisel plow and field cultivator after a crop of spring wheat. Pennycress planting occurred on 15 September 2017 at a rate of 13.5 kg ha^{-1} with a grain drill. Fertilizer was hand broadcasted with 79-34-34 kg ha^{-1} N-P-K on 3 May 2018. An outline of field management practices for pennycress can be found in Table 3-2.

Treatments were three pennycress desiccation dates and controls were mono-cropped soybean and naturally senesced pennycress (Table 3-3). Prior to desiccation, pennycress plants were assessed for phenology and measured for plant height. Defol 5 (Drexel Chemical, Memphis, TN) was applied to pennycress at three intervals beginning on 12 June in 2017 and on 1 June in 2018 at a rate of 11.3 L a.i. ha^{-1}. In 2017, once pennycress had dried, it was harvested using a plot combine and seed was bagged and dried at 65°C for 48 h to a constant mass. Seed yield was adjusted to 80 g kg^{-1} (wt/wt). In 2018, due to untimely precipitation and equipment issues, a 0.25-m^2 area of biomass was hand harvested from each plot prior to desiccation to measure seed yield. Biomass samples were then bagged and dried at 65°C for 48 h to a constant mass. The 2018 samples were destructively processed for seed using a thresher. Seed yield was weighed and adjusted for moisture to 80 g kg^{-1} (wt/wt).

All soybeans were planted using a four-row planter at a rate of 370,658 seeds ha^{-1}. Control soybeans (ASGROW AG1935, MG 1.9) were no-till drilled into plots containing pennycress that had been terminated with glyphosate [N-(phosphonomethyl) glycine] following spring thaw and double-cropped soybeans (ASGROW AG1135, MG 1.1) were planted following pennycress harvest (Table 3-3). In 2017, all soybean plots were sprayed with glyphosate at a rate of 0.842 kg a.i. ha^{-1} on 11 August to maintain a weed-free experiment. In 2018, soybeans were sprayed with glyphosate at a rate of 0.842 kg a.i. ha^{-1} on 27 June. In 2018, soybeans were assessed for soybean aphid (*Aphis glycines* L.) and found to have an infestation above the economic threshold of 273 aphids per plant (Ragsdale et al., 2007) and were sprayed with Warrior II (Lambda-cyhalothrin) (Syngenta International AG, Basel, Switzerland) at a rate of 0.03 kg a.i. ha^{-1} on 22 August. All soybeans were mechanically harvested on 16 November in 2017 and on 22 October in 2018. A summary of soybean field management practices can be found in Table 3-2. Following soybean harvest, seed was bagged and dried at 65°C for 48 h to constant mass. Seed was tested for moisture and yield was adjusted for moisture content to 130 g kg^{-1} (wt/wt).

Seed shatter

In both experiments, pennycress was assessed for seed shatter. During the 2016-2017 growing season, shatter was assessed using methodology previously outlined by Carlson 2018. Shatter was measured by collecting seed loss in shallow plastic weight boats measuring 161 cm^2. Weight boats had small holes poked in the bottom to allow drainage and deter seed germination following shatter. Four containers were placed in each pennycress plot with tops of the containers flush with ground level in the spring following fertilization while pennycress was in the vegetative growth stage. The containers were left in the field and removed just prior to combine harvest to assess seed loss throughout pennycress growth and maturation.

During the 2017-2018 growing season, shatter collection container design was modified to allow for better drainage and more secure placement in the field. This design was similar to those previously used to collect seed shatter (Gesch et al., 2005; Hill et al., 2014) and incorporated a greenhouse pot with an opening measuring 58.0 cm^2. The bottom of the pot was lined with 400 µm mesh and smooth river stones to allow for drainage and keep shattered pennycress seed out of any standing moisture. Three pots were placed in each pennycress plot with tops of the containers flush with ground level following fertilization while pennycress was in the vegetative growth stage. The containers were left in the field and removed just prior to combine harvest to assess seed loss throughout pennycress growth and maturation. Seed from each collection point was removed from the plot and dried at 60°C until it was at constant moisture. To remove chaff and debris, samples were cleaned using sieves that complied with US Standard Testing standards measuring 4750 µm, 2000 µm, and 710 µm. Remaining debris was removed by hand using tweezers. Seed from each plot was weighed to determine shatter losses.

Oil analysis

All seed was tested for oil content. Seed oil content was measured by pulsed nuclear magnetic resonance (NMR) (Bruker Minispec mq-10, Bruker, The Woodlands, TX, USA) using 7 g of seed from each replicated plot (n=4). Prior to NMR analysis, pennycress seed was dried at 130°C for 3h and cooled in a desiccator for 30 min. The

NMR instrument was calibrated with pure pennycress oil and values for oil content are reported as g kg^{-1} (dry wt oil/dry wt seed).

Economic analysis

An assessment of net profit was calculated for each production system in this study: a full season soybean monocrop, a mid-season soybean monocrop, and a mid-season soybean double-cropped with pennycress. Double crop data were pooled over years within each experiment to most accurately represent the value of the system and include both favorable and unfavorable production circumstances. The assessment was conducted in U.S. dollars on a ha^{-1} basis based on market values and recent rates for inputs for pennycress and soybean (National Agricultural Statistics Service, 2018; FINBIN, 2019; United States Department of Agriculture/Foreign Agricultural Service, 2019). Machinery cost estimates were 2018 costs based on University of Minnesota estimates (Lazarus, 2018; FINBIN, 2019). Machinery costs included labor, repairs, fuel and oil, depreciation, and machinery overhead, including interest, insurance, and housing costs. A piecewise breakdown of management expenses can be found in Table 3-5.

Industrial rapeseed (*Brassica napus* L.) oil price was used as a surrogate for pennycress oil price due to similar oil and protein content as the pennycress market is not well established. Once markets for pennycress are established, this may or may not be an accurate representation of the values of those markets. Seed yield was taken directly from experimental data. Input costs assumed that the crop was at an appropriate dry weight leaving the field. Net return was then calculated using Eq. 1.

$$Net\ return = (Oil\ value\ x\ Oil\ yield) - Management\ expenses; \tag{1}$$

Statistical analysis

Parameters of pennycress seed yield, oil content, oil yield, and shatter, as well as soybean seed yield, oil content, and oil yield were analyzed using linear mixed-effect models constructed using the lmer function of the *lme4* package (version 1.1-20) of R 3.5.2 (R Core Team, 2016; Bates et al., 2019), considering treatment and year as fixed effects and blocks as random effects (Table 3-6). Seed shatter data was hetereoscedastic and violated the analysis of variance (ANOVA) assumption of equal variance. Therefore, the seed shatter data was log(x)-transformed for analysis and was back-transformed for presentation. Year was significant across all experimental parameters, except soybean oil yield in Experiment 1, and could not be combined (Table 3-6). Therefore, data were analyzed by experiment as well as year. Analysis of variance ($\alpha = 0.05$) procedures using the anova function of R (R Core Team, 2016) were used to test the effects of seed yield, oil yield, seed shatter, and net return. When significant differences were found ($P < 0.05$), mean separation was conducted using Tukey's Honest Significant Difference (HSD) at $\alpha = 0.05$.

3.4. RESULTS AND DISCUSSION

Experiment 1. Pennycress, soybean, and aggregate oilseed yield as influenced by pennycress desiccation and soybean planting date

2016-2017 growing season

During periods of pennycress (September through June) and soybean (May through October) growth, the temperature was around the 30-year average (Table 3-1). Precipitation levels were near average over pennycress and soybean growth periods.

Despite elevated precipitation over the entirety of the 2016-2017 growing season, there were 31 mm less precipitation than the 30-year average in September, a critical period of pennycress germination (Phippen et al., 2010b; Gesch et al., 2016). Soil moisture is the most limiting factor to pennycress germination. In prior studies, between 25 and 40 mm of water was applied to pennycress plots to facilitate fall germination (Royo-Esnal et al., 2015a; Johnson et al., 2017), and a lack of moisture at planting can directly contribute to low yields (Royo-Esnal et al., 2015a). Despite the deviation from normal, there was a total of 43 mm of rainfall in September, indicating that a lack of precipitation likely didn’t contribute to reduced seed germination or yield loss (Fig. 3-1; Table 3-1). Precipitation was 20.6 mm above the 30-year average in May, when control soybeans were planted, and around average in June when double-cropped soybeans were planted (Tables 3-1 and 3-3). However, precipitation in July totaled only 23 mm, or 76 mm below average, and may have contributed to low soybean yields, especially for late planted soybean (Fig. 3-1; Tables 3-1 and 3-3), which generally yield less than soybean of the same maturity group planted earlier (Eck et al., 1987; Boyer et al., 2015).

Seed yield of soybean, but not pennycress, significantly differed by treatment in 2017 (Fig. 3-1). The highest pennycress yields occurred at the second desiccation date, 12 June, but did not differ from other desiccation treatments or the undesiccated control (Fig. 3-1; Table 3-3). Over the course of the study, pennycress yield averaged 763 kg ha^{-1}, which is consistent with prior literature where pennycress had been desiccated and combine harvested (Eberle et al., 2015; Dose et al., 2017). There was a significant effect of soybean planting date on yield (Fig. 3-1; Table 3-3). While the control soybeans yielded the highest, 2618 kg ha^{-1}, this was not significantly different than soybean planted after the first or second pennycress desiccation dates or the undesiccated control (Fig. 3-1). Much of May 2017 was below the soybean base temperature of 10°C for germination and may have delayed development of the control soybeans, allowing the second and third planting dates, which were planted under more favorable conditions in June, to develop at a comparable rate to the first planting date (Fig. 3-1; Table 3-1). The fourth and fifth soybean planting dates yielded significantly lower than the top performing treatments, which is likely due to late planting (Fig. 3-1; Egli and Cornelius, 2009). It is somewhat surprising that the final soybean planting date did not suffer a yield penalty for late planting despite being planted on the same day as the fourth desiccation treatment (Fig. 3-1; Table 3-3). One reason for this may have been pennycress shatter. Later desiccation dates corresponded to more mature pennycress at application (Table 3-3) meaning that, physiologically, pennycress was more prone to shatter when the desiccant was applied (McIntyre and Best, 1975; Sedbrook et al., 2014; Dorn et al., 2015). Desiccant application may have increased pennycress shatter and subsequent germination prior to soybean planting, leading to resource competition between soybean and pennycress seedlings. Indeed, desiccation date four had significantly higher seed shatter than earlier desiccation dates (Tables 3-3 and 3-7). In related Brassicaceae species such as arabidopsis (*Arabidopsis thaliana* L.), canola (*Brassica napus* L.), and brown mustard (*Brassica juncea* L.), pod shatter increased with maturity as tissues naturally senesced and the exocarp and mesocarp of the seed pod shrunk, increasing pressure on the rigid dehiscence zone, leading to shatter (Spence et al., 1996). This indicates that natural pennycress maturation coupled with chemical desiccation likely accelerated the shattering process. In addition to increased shattering, very little rain fell between 22 June and 10 July, 2017, which encapsulated the early growth stages of the desiccant 3 and 4 soybean plantings (Table 3-3). This, in conjunction with transpiration from the late-desiccated pennycress plants, may have put undue water stress on soybean seedlings. When soybeans were planted after a rye cover crop in North Carolina over a period of below-average precipitation, it was demonstrated that soybean yields were likely decreased from a lack of moisture due to the additional water demands and transpiration of the cover crop (Wells et al., 2016). While the control pennycress treatment had the greatest amount of shatter in 2017, the physical act of shattering may have occurred later due to delayed pennycress maturation relative to the fourth desiccation date (Tables 3-3 and 3-7). Shattered pennycress from the fourth desiccation date may have provided greater moisture competition for soybean at planting compared to the germinated shatter from the control pennycress treatment and, thus, resulted in slightly lower soybean yields (Fig. 3-1; Table 3-7).

Pennycress desiccation treatment had a significant effect on the aggregate seed yield (totaled pennycress and soybean yield) (Fig. 3-1). The first pennycress desiccation date had the highest aggregate seed yield, 3226 kg ha^{-1}, but did not differ from the control soybeans, second desiccation date, or the undesiccated pennycress treatment (Fig. 3-1). While it is promising that a soybean-pennycress double crop can out-yield a soybean monocrop, it is important to consider pennycress maturation. In a study by Cubins et al. (unpublished), it was determined that pennycress physiological maturity did not occur until growth stages 84 to 86, when 40-60% of seed has reached maturity, respectively (Martinelli and Galasso, 2011). Although the first double cropping treatment yielded comparably to other treatments (Fig. 3-1) and within the yield range of other studies that included pennycress desiccation and mechanical harvest (Eberle et al., 2015; Dose et al., 2017), it is likely that the pennycress had not reached physiological maturity as it had only reached growth stage 79 when the desiccant was applied, meaning that only 90% of seed had reached its full size and hadn't begun to ripen (Table 3-3; Martinelli and Galasso, 2011; Cubins et al., unpublished). In 2017, pennycress likely did not reach physiological maturity until the second desiccation date (Table 3-3). The remaining treatments follow the same step-wise trend as the soybean seed yield, which is unsurprising, given that pennycress yield did not perform differently across treatments.

Oil yield patterns followed a similar trend to both pennycress and soybean yield. While oil was not significant across pennycress treatments, pennycress represents a larger proportion of oil yield than it does for seed yield (Fig. 3-2). Pennycress seed contains between 260 and 390 g kg^{-1} (wt/wt) oil content (Clopton and Triebold, 1944; Moser et al., 2009b; Phippen and Phippen, 2013; Isbell et al., 2015; Gesch et al., 2016; Dose et al., 2017) while soybean only contains between 180 and 220 g kg^{-1} (wt/wt) oil content. Over this experimental period, pennycress oil content ranged between 312 and 338 g kg^{-1} (wt/wt) (data not shown). Soybeans planted after the first and second pennycress desiccation date did not perform differently than the control soybean (Fig. 3-2). However, aggregate oil yield for this experimental year was quite low, the highest performing treatments averaged 614 L ha^{-1}. In contrast, soybean alone yielded 507 L ha^{-1} of oil (Fig. 3-2).

2017-2018 growing season

Over the duration of the 2017-2018 growing season, temperatures were consistent with the 30-year average (Table 3-1). However, precipitation accumulated during the pennycress growth period was 63 mm lower than the 30-year average. Much of this lower-than-average precipitation occurred between March and May, encompassing a large portion of the active pennycress growing season. During this period, there were only 70 mm of rain, which is 99 mm less than the same period during the previous year, and 96 mm less than the 30-year average (Table 3-1). A lack of rain in conjunction with low spring temperatures led to a delay in spring pennycress growth to nearly a month later than the previous season. Temperature during the soybean growth period was at or above the 30-year normal and precipitation was 81 mm above the 30-year average (Table 3-1).

Pennycress yielded very poorly during the 2018 harvest, likely due to low soil moisture content as a result of below-average precipitation (Table 3-1; Johnson et al., 2015). It is typical for more than half of pennycress to germinate in the spring once soil reaches the base temperature threshold of -2.5°C (Royo-Esnal et al., 2015a), and low temperature and moisture conditions through April likely influenced low seasonal yields as well (Fig. 3-1). Pennycress yields did not differ across treatments and averaged only 154 kg ha^{-1}. Considering that pennycress is projected to be profitable for a land owner at yields of at least 1685 kg ha^{-1} (John Sedbrook, personal communication), this is experimental site-year provided evidence that failures in this system can occur. In a year with poor spring stands, it may be more beneficial for a producer to terminate pennycress in similar timing and manner to other winter cover crops and plant the full-season summer annual rather than a double crop. This would allow the producer to reap the benefits of the winter annual cover without decreasing the summer annual growing season.

During the soybean growth period, there was no lack of moisture, in fact, there was 56.6 mm more rain than the 30-year average. The mono-cropped soybean treatment yielded most prolifically, averaging 3336 kg ha^{-1}. Despite favorable growing conditions over the soybean growing season, double-cropped soybean yielded poorly averaging only 1142 kg ha^{-1} (Fig. 3-1). The double-cropped soybean treatments yield 67% lower than the Stevens county average of 3477 kg ha^{-1} (National Agricultural Statistics Service, 2019). This season is a good example of the risk incurred when double cropping pennycress with soybean. As a winter cover crop, pennycress can be both beneficial and harmful to the subsequent soybean crop by either capturing soil moisture and creating a more beneficial germination environment (Unger and Vigil, 1998), or, conversely, a lack of water could lead to a droughty conditions during soybean germination (Unger and Vigil, 1998; Johnson et al., 2015; Royo-Esnal et al., 2017). Later-than-average pennycress maturation due to a late spring caused the double-cropped soybeans to be planted over a month later than the control soybeans (Table 3-3). Soybean planted in mid-June as opposed to mid-May are noted to produce fewer pods per plant and side branch, and yield 400 kg ha^{-1} less seed (Cox et al., 2008) while losing as much as 328 kg ha^{-1} $week^{-1}$ that planting is delayed

(De Bruin and Pedersen, 2008). Additionally, the lower-than-average precipitation during September, when much of soybean reproductive growth occurred, and lower-than-average temperatures during late September through October likely depressed yield. One study found that water stress during soybean reproductive growth stages diminished yields between 9 and 65%, depending on the duration of drought and soybean growth stage under drought conditions (Eck et al., 1987). Unlike in 2017, pennycress seed shatter due to desiccation and subsequent seedling competition did not appear to have an effect on soybean in 2018 despite significantly higher shatter values for the final pennycress desiccation date and undesiccated pennycress control compared to other treatments (Fig. 3-1; Table 3-7).

Pennycress oil yield did not differ across treatments while soybean oil yield did. Pennycress oil yield averaged 249 L ha^{-1}. Oil yield followed a similar pattern to seed yield where the soybean control plot yielded greatest (Fig. 3-2). There were significant differences between soybean treatments. The soybean mono-crop performed significantly better than all soybeans double-cropped with pennycress (Fig. 3-2). Both aggregate seed and oil yield mimicked the pattern observed with soybean yield where the mono-cropped treatment yield significantly greater seed and oil yield in comparison to the double-cropped treatments, which did not yield significantly different from each other (Figs. 3-1 and 3-2). Overall, this experimental year demonstrates the risk of double-cropping pennycress with soybean in the northern Corn Belt, and potentially other regions with short growing seasons.

While year significantly influenced seed yield, oil yield, and seed shatter throughout Experiment 1 (Table 3-6), there were trends across both years. Pennycress yields were low, but consistent across treatments indicating that spring management (*i.e.*, timing of desiccant application and harvest) may not have affected yield greatly. Dose et al., (2017) determined that a longer maturation period favored higher yields, which may indicate that an earlier pennycress planting date to increase the amount of pennycress vegetation prior to winter may result in yield gains. While it is often not possible to accurately predict when the first frost will arrive, planting pennycress earlier rather than later in the fall may be a good strategy to encourage fall, rather than spring, germination when light availability and soil temperatures are high (Best and McIntyre, 1975; Phippen et al., 2010b; Royo-Esnal et al., 2017).

Experiment 2. Pennycress, soybean, and aggregate oilseed yield as influenced by pennycress desiccation and soybean maturity group

2016-2017 growing season

Over the course of the pennycress and soybean growing seasons, temperature remained close to the 30-year average, while precipitation deviated more dramatically. The winter preceding the 2017 pennycress harvest was slightly warmer than usual, where November 2016 and February 2017 were 6.1 and 5.8°C warmer than the average temperature, respectively (Table 3-4). Overall, the pennycress growing season accumulated 83.7 mm more precipitation than an average year while the soybean growth period accumulated 19.3 mm less precipitation than an average year (Table 3-4).

There was no significant variation in yield between pennycress treatments (Fig. 3-3). This is likely due to the range of pennycress growth stages that were captured during the experiment. Phenology spanned stages 84, 85, and 86 when seed was 40%, 50% , and 60% mature with desiccant being applied within five days to capture those growth stages (Table 3-3; Martinelli and Galasso, 2011). Incidentally, these growth stages corresponded with the range found to maximize seed yield in hand harvested plots by Cubins et al. (unpublished), which may partially explain the lack of yield variation (Fig. 3-3). The average pennycress yield was 1090 kg ha^{-1} (Fig. 3-3), which is consistent with other studies that reported maximum yield values of 1100 and 1110 kg ha^{-1} when using a desiccant and plot combine to harvest pennycress (Eberle et al., 2015; Dose et al., 2017).

Unlike Experiment 1, Experiment 2 utilized two soybean maturity groups and only two soybean planting dates, one for each maturity group, to assess the difference between mono-cropped and double-cropped soybean (Table 3-3). Mono-cropped soybean had the greatest yield, averaging 4575 kg ha^{-1}, while double-cropped soybean treatments, which were all planted on the same day, yielded similarly to each other, averaging 3799 kg ha^{-1} (Fig. 3-3; Table 3-3). Part of this yield differential may have been due to differences in maturity group; the mono-cropped and double-cropped soybeans were in maturity groups 1.9 and 1.1, respectively. While maturation is greatly dependent on temperature and photoperiod, lower maturity groups spend less time in the vegetative stage before transitioning to reproductive growth (Hartwig, 1970). A lower maturity group in conjunction with late planted beans likely reduced time for vegetative growth, which corresponds to smaller plants with fewer and smaller leaves (*i.e.*, lower leaf area index), leading to less light capture and smaller seed set (Malone et al., 2002; Holshouser, 2003; Caviglia et al., 2011). Soybeans must reach a leaf area index between 3.5 and 4 to reach maximal yield goals, meaning that there are nearly four full layers of foliage throughout the soybean canopy (Malone et al., 2002; Holshouser, 2003). To offset the risk of soybeans not capturing enough light for photosynthetic activity due to low maturity group or late planting date, higher planting rates are recommended (Holshouser, 2003). Another aspect to consider are the differences in plant maturation. Maturity group was staggered to decrease the risk and yield loss incurred with double cropping. However, the late planted soybeans were not able to reach full maturity before the first killing frost (data not shown). A lower maturity group may have allowed soybeans to reach full maturity prior to the first frost (Mourtzinis and Conley, 2017). Despite depressed soybean yield, aggregate seed yield of the double cropped treatments did not perform differently than the mono-cropped soybean seed yield.

Overall, shatter loss increased with desiccation date as pennycress was more mature when desiccation occurred (Tables 3-3 and 3-7). The greatest shatter was seen in the pennycress control treatment, 446 kg ha^{-1}, but did not have significantly more shatter than the first and third desiccation treatments (Tables 3-3 and 3-7). It is somewhat surprising that seed shatter did not lead to decreased seed yield in pennycress (Fig. 3-1) as might be expected with the increased shatter at later treatment dates (Table 3-3). Pennycress shatter did not appear to affect soybean yield (Figs. 3-1 and 3-3, Table 3-7).

Oil yield followed the same trend as seed yield for both pennycress and soybean (Figs. 3-3 and 3-4). Oil yield was not significant across pennycress treatments (Table 3-

3), likely due to the achievement of physiological maturity by all treatments, after which seed oil content plateaus (Cubins et al., unpublished). Soybean oil yield differed only between monocrop and double crop treatments, where mono-cropped soybean and double-cropped soybean yield averaged 893 and 670 L ha^{-1}, respectively. As expected, pennycress accounted for a proportionally greater amount of oil yield than seed yield and averaged 359 L ha^{-1} at this site-year across treatments (Figs. 3-3 and 3-4). Aggregate double-cropped oil yield was greater than oil yield of the soybean monocrop (Fig. 3-4).

2017-2018 growing season

During the 2017-2018 growing season, February and April were 4.2 and 6.9°C cooler than the 30-year average temperatures, respectively (Table 3-4). These persistent, cool temperatures led to a late spring, delaying pennycress growth to one month later than what is typical (Johnson et al., 2015). The pennycress growing season accumulated 113 mm less precipitation than the 30-year average, however, much of this occurred over the winter when cold temperatures impeded pennycress growth (Table 3-4). The soybean growing season accumulated 96 mm more rainfall than an average year, which ensured adequate water availability during critical periods of soybean growth (Eck et al., 1987).

The 2018 harvest season showed much more variation in pennycress yield than the 2017 harvest season. This is likely due to the larger span of growth stages that were encapsulated over the experimental period (Table 3-3). The first desiccation occurred when seed was at growth stage 78, or when it had reached 80% of its full size and had not begun to ripen (Table 3-3; Martinelli and Galasso, 2011). This corresponded to a very low yield, only 121 kg ha^{-1}, likely due to immaturity at desiccation (Cubins et al., unpublished). The third desiccation date had the highest pennycress yields, averaging 1683 kg ha^{-1}, which is among the highest seed yields reported for pennycress (Carr, 1993; Johnson et al., 2017). This desiccation period corresponded with 40% ripened seed, one of the targeted growth stages for optimal yield (Table 3-3; Martinelli and Galasso, 2011; Cubins et al., unpublished). Pennycress samples during this year of study were hand harvested due to mechanical issues. Hand harvesting corresponds to less plot disturbance than if the plots were direct-combine harvested (Sintim, 2014). While it is valuable to consider the yield potential of the selected wild type used in this experiment, it cannot be directly compared to the 2016-2017 experimental period where pennycress was mechanically harvested (Fig. 3-3). The second desiccation treatment and undesiccated pennycress control yielded similarly to each other (Fig. 3-3). It was expected that the yield of the second desiccation treatment would be poor due to immaturity, however, it also had the high values for shatter in the experiment, losing 986 kg ha^{-1} of seed due to shatter (Table 3-7). The undesiccated pennycress control yielded similarly to the second desiccation treatment (Fig. 3-3). It is unclear why the third desiccation had less shatter than the second desiccation as both treatments occurred within close temporal proximity and would have been exposed to similar environmental factors (Tables 3-3 and 3-7). Severe lodging within this site-year led to extremely uneven plot maturation (data not shown), so it is possible that lodged plants were not as mature and did not shatter as readily or were protected from environmental disturbance by vegetation in the upper canopy of the pennycress stand.

Soybean yield was more variable across treatments than was seen in the previous year (Fig. 3-3). The mono-cropped soybean was the highest yielding, but was not significantly different from the soybean planted following the first and third pennycress desiccation treatments or the undesiccated pennycress control. The soybean planted following the second pennycress desiccation had the lowest yields, which may have been due to competition from germinated pennycress seed shatter, as this desiccation date exhibited the highest rate of shatter loss (Fig. 3-3; Table 3-7). There was a ten-day gap between desiccation and harvest (Table 3-3). This, coupled with warmer-than-average temperatures following desiccation (Table 3-4), may have accelerated pennycress maturation prior to harvest, making it more vulnerable to early shatter and germination (personal observation). Warmer-than-average temperatures and above-average precipitation likely contributed to the success of the double crop in this season despite the late onset of spring (Table 3-4; Clopton and Triebold, 1944; Johnson et al., 2015; Royo-Esnal et al., 2017).

Aggregate seed yield was highest for the third desiccation treatment, averaging 5090 kg ha^{-1}, exceeding only than the first pennycress desiccation treatment and the soybean monocrop (Fig. 3-3). In comparison to the mono-crop, the top performing double-cropped treatments yielded 12% more oilseed than soybean alone (Fig. 3-3). In other pennycress-soybean double crop studies, the difference is often much greater. Johnson et al. (2017) found that annual seed yield increased by 48-102% when pennycress and soybean were totaled despite an 18-30% yield depression in soybean.

Oil yield followed the same trend across pennycress treatments that was seen in seed yield (Figs. 3-3 and 3-4). The earliest desiccation date resulted in the lowest oil yield, due to the immaturity of pennycress at desiccation (Table 3-3). Similar oil content across the other three pennycress treatments, averaging 314 g kg^{-1} (wt/wt) (data not shown), likely lead to a higher contribution by seed yield resulting in differences in oil yield. While pennycress oil yield was not different across treatments at in 2017, the significant differences indicated in 2018 demonstrate that harvest timing can significantly affect the oil content of pennycress. The primary agronomic product of pennycress is oil (Moser et al., 2009a; Moser, 2012), and it can be significantly affected by both seed yield and oil content at harvest. Soybean oil yield was significantly higher for the mono-cropped soybeans in comparison to the double-cropped soybeans (Fig. 3-4). This indicates that, despite yielding similarly, there were differences in oil accumulation. Indeed, the double-cropped soybeans were not able to reach full maturity before the first killing frost (personal observation). This is one indicator that the soybean maturity group used may not have been appropriate for use in this double cropping scenario. The double-cropped soybeans accumulated 197 g kg^{-1} (wt/wt) of oil compared to the control soybeans, which accumulated 213 g kg^{-1} (wt/wt) of oil (data not shown). It is possible that this was due to the lower maturity group chosen for the double-cropped treatments. In 2018, aggregate oil yield for the third desiccation date was higher than all other treatments and coincided with pennycress physiological maturity (Fig. 3-3; Table 3-3). As pennycress makes up a larger proportion of aggregate oil yield than it does in seed yield, this treatment outperformed the mono-cropped soybean.

Overall, Experiment 2 demonstrates how a double crop can outperform a monocrop (Figs. 3-3 and 3-4). While soybean yields were depressed by the addition of a double crop in most scenarios, aggregate seed yield was the same, if not greater, when pennycress was added to the cropping system as was observed by Johnson et al. (2017). Although soybeans did not reach full maturity prior to the first frost over the 2016-2017 or 2017-2018 growing season, they were able to reach full seed fill. One strategy to reduce the risk of immature soybean when the first frost occurs is to choose a shorter season maturity group or to relay crop, rather than double crop, soybean.

Economic analysis

At first assessment, it appears that the pennycress-soybean double crop is not an economically beneficial cropping system (Table 3-8). The full-season soybean, maturity group 1.9, had the highest, and only positive, net return, \$117.49 ha^{-1}, based on the experimental data (Figs. 3-3; Tables 3-5 and 3-8). In comparison, the mid-season soybean, maturity group 1.1, had a net return of -\$65.50 ha^{-1}, while the mid-season soybean double-cropped with pennycress had a net return of -\$638.42 and -\$239.50 ha^{-1} for experiments 1 and 2, respectively (Tables 3-5 and 3-8). Much of this loss was incurred due to the amount of labor and equipment needed to facilitate double crop management; equipment related expenses were over 40% higher than for a monocrop (Table 3-5). Experimental conditions weren't ideal for double cropping in each site-year of both experiments, which is one of the risks incurred when implementing an alternative cropping system (Tables 3-1 and 3-4). A major limiting resource for double cropping is water availability (Clopton and Triebold, 1944; Johnson et al., 2015; Royo-Esnal et al., 2017). In both years of both experiments, there was lower-than-average precipitation during the late spring or early summer, which may have contributed to lower-than-expected crop performance (Tables 3-1 and 3-4).

The pennycress line used in these trials must also be considered. The current experimental line 'MN106' is a selected wild type and retains many weedy characteristics such as seed shatter (Best and McIntyre, 1975; Carlson, 2018), uneven maturation (Dorn et al., 2015), and seed dormancy (Saini et al., 1987; Dorn et al., 2015; Gesch et al., 2016), which further confounds the development of agronomic best management practices. The average yields across all pennycress treatments was 722 kg ha^{-1}, which is typical for the current surrogate pennycress line when mechanically harvested (Eberle et al., 2015; Dose et al., 2017). This average includes the exceptionally low yields from Experiment 1 in 2018 of only 154 kg ha^{-1} (Fig. 3-1). The lowest yields reported previously under similar experimental conditions (*i.e.*, desiccation and mechanical harvest) were 200 and 220 kg ha^{-1} (Eberle et al., 2015; Dose et al., 2017), indicating the observed yields may have been exceptionally low, even for the shatter-prone line. While this may not currently be as profitable as a monocrop system, it is important to keep in mind that this double-crop system has not yet been optimized for production conditions in the Upper Midwest. Further optimization may improve the economic risk profile for the pennycress-soybean double crop system.

Pennycress likely contributes to farm profitability in more subtle manners because of its dual purpose as a cash crop and a cover crop. The current two-year corn-soybean rotation experiences about 75% of its annual nitrate leaching between April and June due to land left fallow over the winter and spring (Randall and Vetsch, 2005), indicating there is excess nitrogen available to a crop in the shoulder season. Indeed, Ott 2018 found that there was a 20 kg ha^{-1} reduction in nitrate levels in fields planted with pennycress in comparison to a fallow control. Although the pennycress plots in this experiment were fertilized, a prior study suggests that no fertilizer may need to be added due to the success of pennycress as a nutrient-scavenging crop (Johnson et al., 2017). However, pennycress nitrogen fertility still needs to be optimized (Cubins et al., unpublished). In decreasing nitrogen loss from a field, fewer nutrient inputs may be required over time.

Soybean maturity group should also be carefully considered due to issues with maturation at both sites (data not shown). Soybean yield potential was reduced in the double crop system, this was especially evident in Experiment 1 in 2018 and Experiment 2 in 2017, and directly corresponded to soybean yield depression (Figs. 3-1 and 3-3). While some soybean yield decline is expected with a later planting date (Boyer et al., 2015), there appears to be room to optimize soybean genetics for double-cropping with pennycress in the Upper Midwest, to ensure maturation before the end of the season. One of the purposes of this study was to assess the limitations of a pennycress-soybean double crop, and, in this instance, soybean maturity group was one of them. This indicates that soybean maturity group should be adjusted further for the double-cropped beans to fully develop oil before harvest and will further enhance the profitability of this system.

3.5. CONCLUSIONS

The primary goal of this research was to explore known limitations of the pennycress-soybean double cropping system including aggregate oilseed production as a function of pennycress desiccation and soybean planting date, pennycress seed shatter, and economic profitability. Pennycress seed and oil yields were the greatest when pennycress reached physiological maturity and when desiccant was applied close to physiological maturity, this was most evident in Experiment 2 in 2018. Soybean yields were greatest when an appropriate maturity group was planted and precipitation was abundant; the mono-cropped soybeans performed the best given these constraints. While the double cropping scenario did not perform particularly well under these experimental conditions, these experiments provided useful information to consider more carefully in future research. In optimizing this alternative cropping system, economically, pennycress double-cropped with soybean should become more profitable and likely more enticing to growers in the Upper Midwest.

3.6. TABLES AND FIGURES

Table 3-1. Monthly climate data for Experiment 1 at Morris, MN over the 2016-2017 and 2017-2018 growing seasons.

	2016-2017				2017-2018			
Month	†Mean air temperature °C	‡Departure from normal °C	Accumulated precipitation mm	Departure from normal mm	Mean air temperature °C	Departure from normal °C	Accumulated precipitation mm	Departure from normal mm
Sep	16.7	1.7	42.9	-30.8	17.1	2.1	104.7	31.0
Oct	9.6	2.2	87.1	22.8	8.7	1.3	68.6	4.3
Nov	4.8	6.1	42.2	14.8	-1.4	-0.1	11.9	-15.5
Dec	-9.2	0.2	32.5	14.2	-9.2	0.2	6.9	-11.4
Jan	-9.1	3.1	13.2	-5.6	-10.7	1.5	3.8	-15.0
Feb	-2.8	6.6	11.4	-6.6	-13.0	-3.6	21.3	3.3
Mar	-0.9	1.4	11.7	-24.6	-1.8	0.5	20.3	-16.0
Apr	7.6	1.0	65.0	6.3	1.4	-5.2	24.4	-34.3
May	13.5	-0.3	92.5	20.6	17.6	3.8	26.2	-45.7
Jun	19.8	0.8	101.1	-0.8	21.0	2.0	138.2	36.4
Jul	22.0	0.7	22.6	-76.0	21.4	0.1	143.0	44.4
Aug	18.5	-1.6	175.0	90.4	20.5	0.4	96.8	12.2
Sep	17.1	2.1	104.7	31.0	16.0	1.0	49.5	-24.2
Oct	8.7	1.3	68.6	4.3	5.1	-2.3	76.2	11.9

†Mean air temperature and accumulated precipitation were recorded from a weather station at the Swan Lake Research Farm, Morris, MN.

‡ Calculated departure from the 1981-2010 30-year average temperature and accumulated precipitation using data collected at the West Central Research and Outreach Center, 18 km away from the research site (NOAA/NCEI, 2017).

Table 3-2. Summary of field management operations for pennycress and soybean in Experiments 1 and 2 over the 2016-2017 and 2017-2018 growing seasons.

	Experiment 1		Experiment 2	
	2016-2017	2017-2018	2016-2017	2017-2018
Preceding crop	Spring wheat	Spring wheat	Alfalfa	Spring wheat
Fertilization				
Method	Broadcast	Broadcast	Hand broadcast	Hand broadcast
Rate (kg ha^{-1} N-P-K)	79-34-34	79-34-34	79-34-34	79-34-34
Pennycress				
Planting date	13 Sept 2016	16 Sept 2017	27 Sept 2016	15 Sept 2017
Planting method	No-till drill	No-till drill	Broadcast	No-till drill
Planting rate (kg ha^{-1})	11.2	11.2	11.2	13.5
Harvest method	Combine	Combine	Combine	Hand harvest
Soybean monocrop				
Variety	ASGROW AG1135	ASGROW AG1135	ASGROW AG1935	ASGROW AG1935
Maturity group	1.1	1.1	1.9	1.9
Planting rate (seeds ha^{-1})	420,078	420,078	370,658	370,658
Harvest date	12 Oct 2017	22 Oct 2018	16 Nov 2017	22 Oct 2018
Soybean double crop				
Variety	ASGROW AG1135	ASGROW AG1135	ASGROW AG1135	ASGROW AG1135
Maturity group	1.1	1.1	1.1	1.1
Planting rate (seeds ha^{-1})	420,078	420,078	370,658	370,658
Harvest date	25 Oct 2017	31 Oct 2018	16 Nov 2017	22 Oct 2018
Herbicide application				
Herbicide	Trifluralin	Trifluralin	Glyphosate	Glyphosate
Date	11 Sept 2016	14 Sept 2016	11 Aug 2017	27 Jun 2018
Rate (kg a.i. ha^{-1})	1.1	1.1	0.842	0.842
Insecticide application				

Insecticide	---	---	---	Warrior II
Date	---	---	---	22 Aug 2018
Rate (kg a.i. ha^{-1})	---	---	---	0.03

Table 3-3. Outline of pennycress and soybean treatment differences in terms of pennycress desiccation date, phenology at desiccation, and harvest date and soybean maturity group and planting date.

	Pennycress						Soybean		
Treatment	Desiccation date		Phenology at desiccation		Harvest date		Maturity group	Planting date	
Experiment 1	2017	2018	2017	2018	2017	2018		2017	2018
Soybean control	---	---	---	---	---	---	1.1	11 May	22 May
Desiccant 1	5 Jun	15 Jun	79	78	15 Jun	29 Jun	1.1	15 Jun	29 Jun
Desiccant 2	12 Jun	20 Jun	86	81	19 Jun	29 Jun	1.1	19 Jun	29 Jun
Desiccant 3	15 Jun	22 Jun	87	84	20 Jun	2 Jul	1.1	20 Jun	2 Jul
Desiccant 4	19 Jun	26 Jun	88	87	23 Jun	5 Jul	1.1	23 Jun	5 Jul
Pennycress control	----	---	---	---	23 Jun	5 Jul	1.1	23 Jun	5 Jul
Experiment 2									
Soybean control	----	---	---	---	---	---	1.9	31 May	1 Jun
Desiccant 1	12 Jun	1 Jun	84	78	19 Jun	22 Jun	1.1	23 Jun	22 Jun
Desiccant 2	14 Jun	12 Jun	85	81	19 Jun	22 Jun	1.1	23 Jun	22 Jun
Desiccant 3	16 Jun	15 Jun	86	84	19 Jun	22 Jun	1.1	23 Jun	22 Jun
Pennycress control	---	---	---	---	19 Jun	22 Jun	1.1	23 Jun	22 Jun

Table 3-4. Monthly climate data for Experiment 2 at Rosemount, MN over the 2016-2017 and 2017-2018 growing seasons.

	2016-2017				2017-2018			
Month	†Mean air temperature °C	‡Departure from normal °C	Accumulated precipitation mm	Departure from normal mm	Mean air temperature °C	Departure from normal °C	Accumulated precipitation mm	Departure from normal mm
Sep	18.1	2.1	132.8	40.6	17.9	1.9	42.4	-49.8
Oct	11.1	2.2	62.2	-10.4	9.6	0.7	98.6	26.0
Nov	6.2	6.1	45.2	-8.1	-0.6	-0.7	1.8	-51.5
Dec	-7.1	1.1	24.1	-6.9	-8.2	0.0	8.4	-22.6
Jan	-7.6	3.1	51.8	25.4	-11.1	-0.4	24.9	-1.5
Feb	-1.9	5.8	16.0	-7.1	-11.9	-4.2	28.2	5.1
Mar	-0.8	-0.3	16.0	-42.4	-1.4	-0.9	23.1	-35.3
Apr	9.0	1.1	115.6	41.4	1.0	-6.9	50.3	-23.9
May	13.5	-0.8	182.4	79.8	18.6	4.3	108.7	6.1
Jun	20.5	0.9	91.4	-28.5	21.5	1.9	154.4	34.5
Jul	22.3	0.4	138.7	24.4	22.0	0.1	111.0	-3.3
Aug	18.9	-1.8	128.8	8.7	21.1	0.4	102.1	-18.0
Sep	17.9	1.9	42.4	-49.8	17.4	1.4	157.2	65.0
Oct	9.6	0.7	98.6	26.0	6.2	-2.7	90.9	18.3

†Mean air temperature and accumulated precipitation were recorded from a weather station at the Rosemount Research and Outreach Center, Rosemount, MN.

‡Calculated departure from the 1981-2010 30-year average temperature and accumulated precipitation using data collected at the Rosemount Research and Outreach Center (NOAA/NCEI, 2017).

Table 3-5. Soybean (Soy), pennycress (PC), and soybean-pennycress double crop (DC) estimated annual production expenses based on management during the 2016-2017 and 2017-2018 growing seasons.

Item	Soy	PC	DC
Seed costs (\$ ha^{-1})	111.20	61.77	172.97
Fertilizer application (\$ ha^{-1})	50.95	185.48	185.48
Chemical application (\$ ha^{-1})			
Weed control	37.07	37.07	74.13
Pest control	0.90	0.00	0.90
Desiccant	0.00	17.05	17.05
†Machinery (\$ ha^{-1})			
Chisel plow	27.73	27.73	27.73
Field cultivator	15.74	15.74	15.74
No-till drill	62.22	62.22	124.44
Boom sprayer	22.68	22.68	45.37
Combine	94.76	94.76	189.53
Grain cart	59.03	59.03	118.07
Total estimated cost (\$ ha^{-1})	482.28	583.53	971.41

†Costs includes fuel, lubricants, repairs and maintenance, labor, and power and implement depreciation

Table 3-6. Mixed-model analysis of variance for study parameters for treatment (T), year (Y), and interaction (TxY).

		Pennycress				Soybean		
Experiment	†Fixed effects	Seed yield	Oil content	Oil yield	¶Shatter	Seed yield	Oil content	Oil yield
Morris	T	‡ns	§***	ns	***	***	***	***
	Y	***	***	***	***	***	***	ns
	TxY	ns	***	*	***	***	***	***
Rosemount	T	***	***	***	***	***	***	***
	Y	*	***	***	***	***	***	***
	TxY	***	***	***	***	ns	***	ns

†Maximum-likelihood comparisons guided model simplification.

‡ns refers to parameters that were not significant for the given fixed effect.

§*, **, and *** refers to parameters with p-values of 0.05, 0.01, and 0.001, respectively, for a given fixed effect.

¶Shatter refers to amount of seed lost due to environmental conditions between vegetative growth and harvest.

Table 3-7. Pennycress seed loss due to shatter across desiccation treatments (Des 1-4) and the undesiccated control treatment (PC). Seed shatter values within an experiment and year sharing a letter are not statistically different from each other based on Tukey's HSD ($P < 0.05$).

	2017					2018				
	Des 1	Des 2	Des 3	Des 4	PC	Des 1	Des 2	Des 3	Des 4	PC
	Seed shatter (kg ha^{-1})									
Experiment 1	621.4b	331.5b	400.4b	1178.3a	1228.5a	2.2b	1.7b	16.4b	109.1a	131.8a
Experiment 2	213.2ab	181.8b	225.9ab	---	446.1a	312.6c	986.3a	587.5bc	---	850.3ab

Table 3-8. Full season soybean (FS), mid-season soybean (MS), and soybean-pennycress double crop (DC) aggregate oil yield and projected gross and net return based on current markets for soybean and rapeseed oil. Gross and net return values within a row sharing a letter are not statistically different from each other based on Tukey's HSD (*P* < 0.05).

			DC	
Item	FS	MS	Experiment 1	Experiment 2
Aggregate oil yield (L ha^{-1})	858	594	428	981
Gross return (\$ ha^{-1})	593.81 ab	410.82 b	327.03 b	725.95 a
Crop subsidy (\$ ha^{-1})	5.96	5.96	5.96	5.96
Total costs (\$ ha^{-1})	482.28	482.28	971.41	971.41
Net return (\$ ha^{-1})	117.49 a	-65.50 ab	-638.42 c	-239.50 b

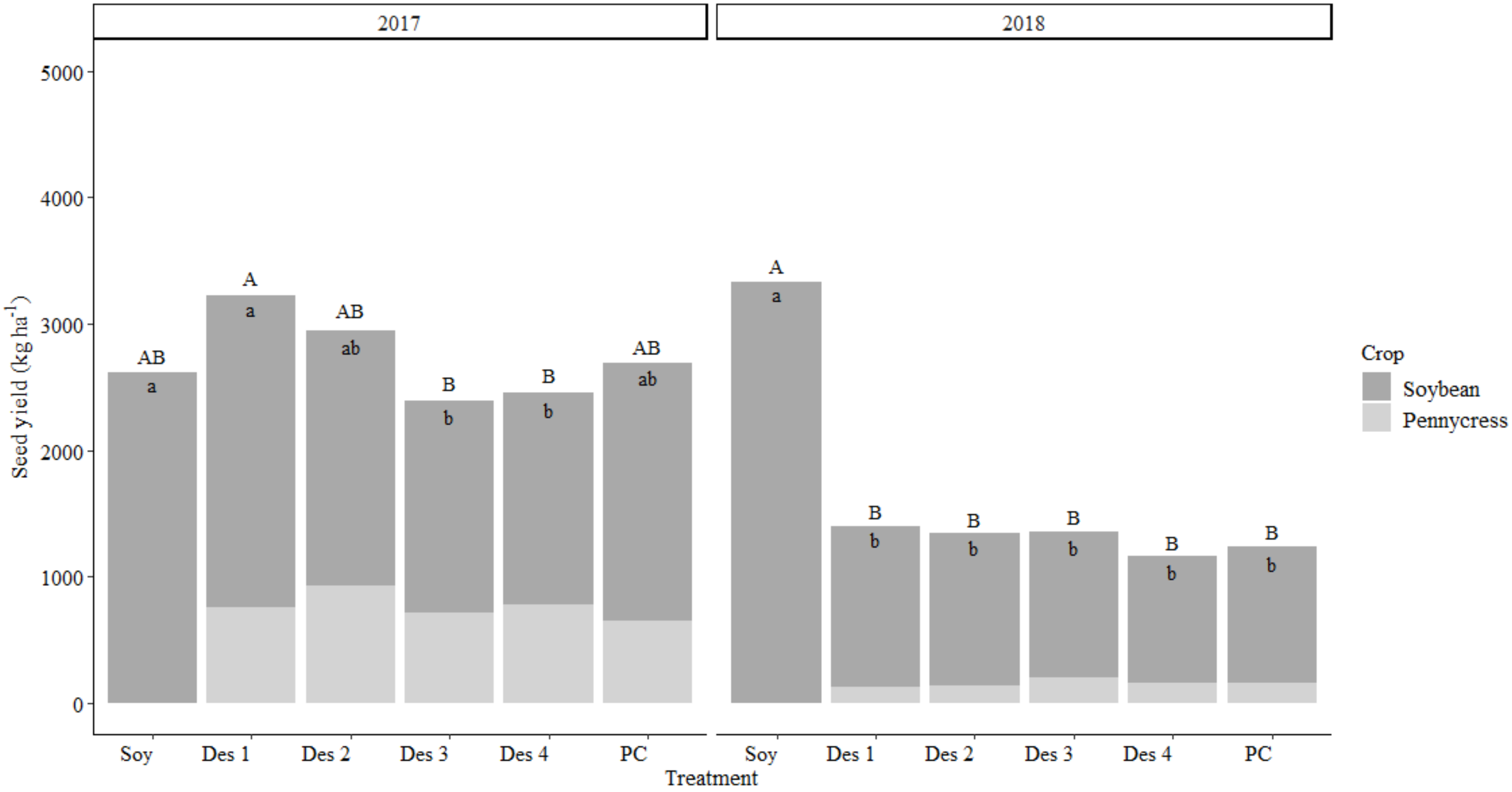

Figure 3-1. Seed yield of pennycress and soybean in Experiment 1 over the 2017 and 2018 harvest seasons. Treatments are the mono-cropped control soybean (Soy), pennycress desiccation date (Des 1-4), and the undesiccated pennycress control (PC). Mean values sharing the same lowercase letter within a year and crop type and bars of aggregate seed yield that share capital letters within a year are not significantly different from each other. Mean values that lack lowercase letters within a year and crop type were not significantly different from each other. All mean separation values are based on Tukey's HSD ($P < 0.05$).

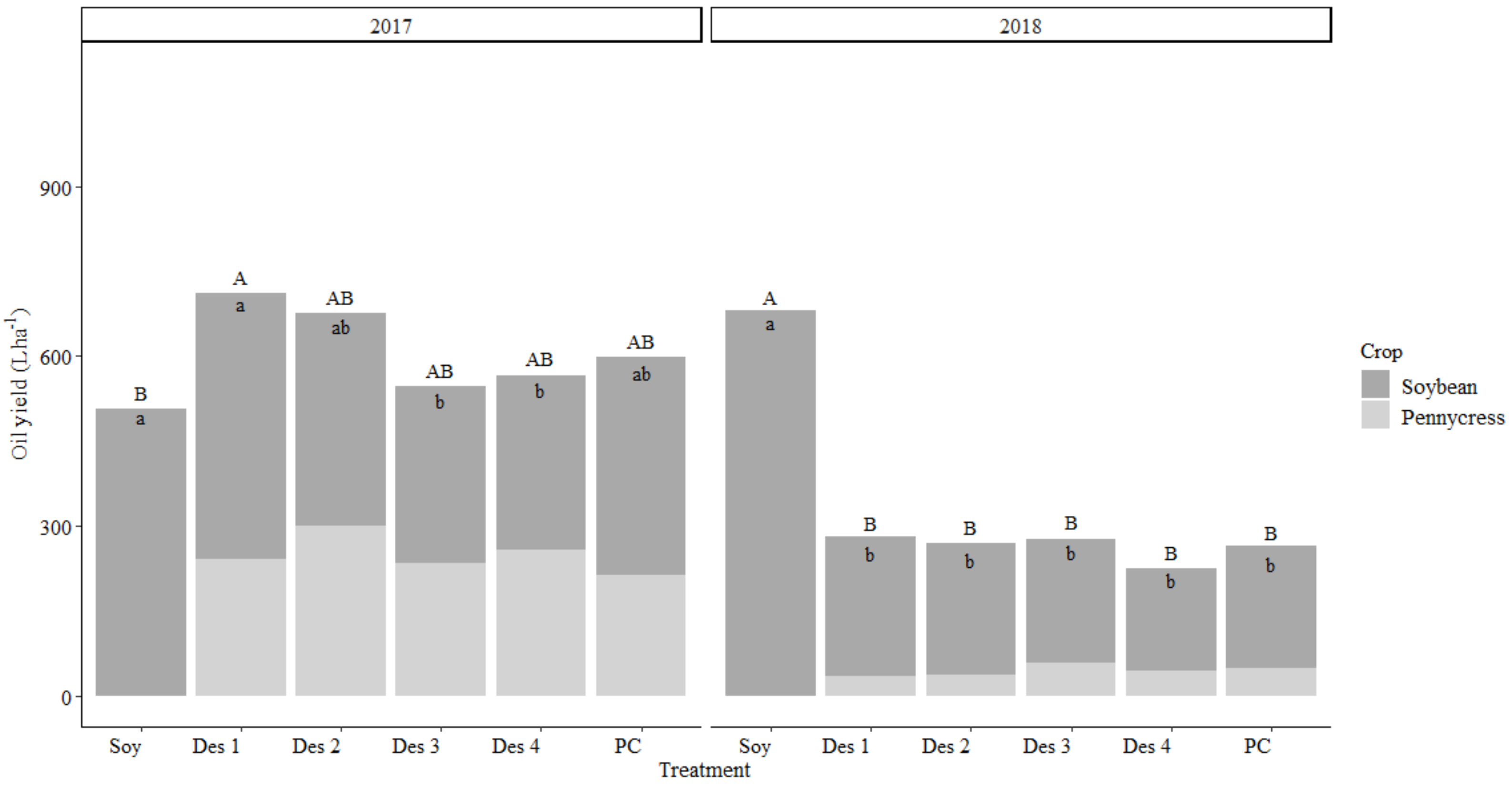

Figure 3-2. Calculated oil yield of pennycress and soybean in Experiment 1 over the 2017 and 2018 harvest seasons. Treatments are the mono-cropped control soybean (Soy), pennycress desiccation date (Des 1-4), and the undesiccated pennycress control (PC). Mean values sharing the same lowercase letter within a year and crop type and bars of aggregate seed yield that share capital letters within a year are not significantly different from each other. Mean values that lack lowercase letters within a year and crop type were not significantly different from each other. All mean separation values are based on Tukey's HSD ($P < 0.05$).

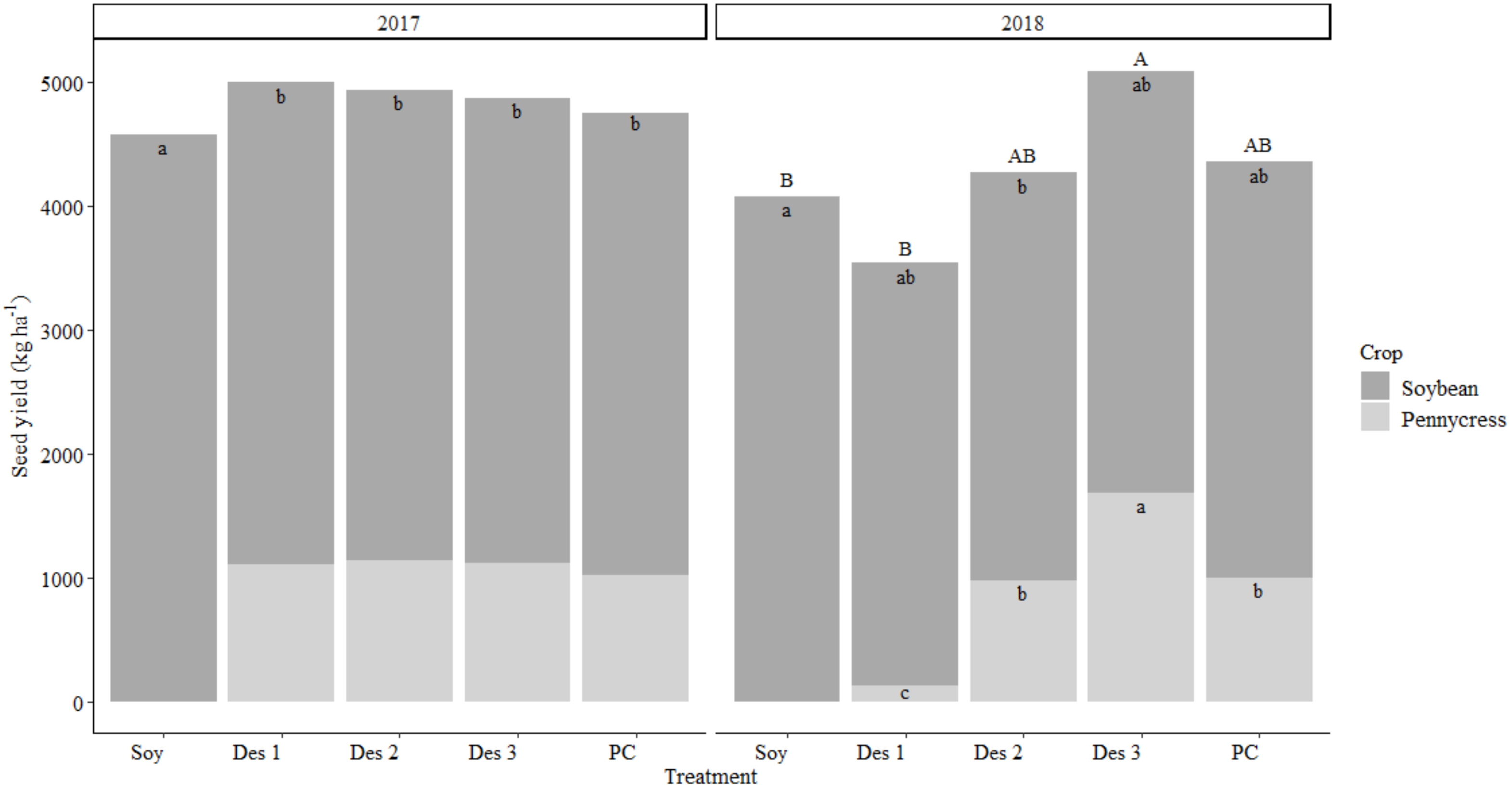

Figure 3-3. Seed yield of pennycress and soybean in Experiment 2 over the 2017 and 2018 harvest seasons. Treatments are the mono-cropped control soybean (Soy), pennycress desiccation date (Des 1-3), and the undesiccated pennycress control (PC). Mean values sharing the same lowercase letter within a year and crop type and bars of aggregate seed yield that share capital letters within a year are not significantly different from each other. Mean values that lack lowercase letters within a year and crop type were not significantly different from each other. All mean separation values are based on Tukey's HSD ($P < 0.05$).

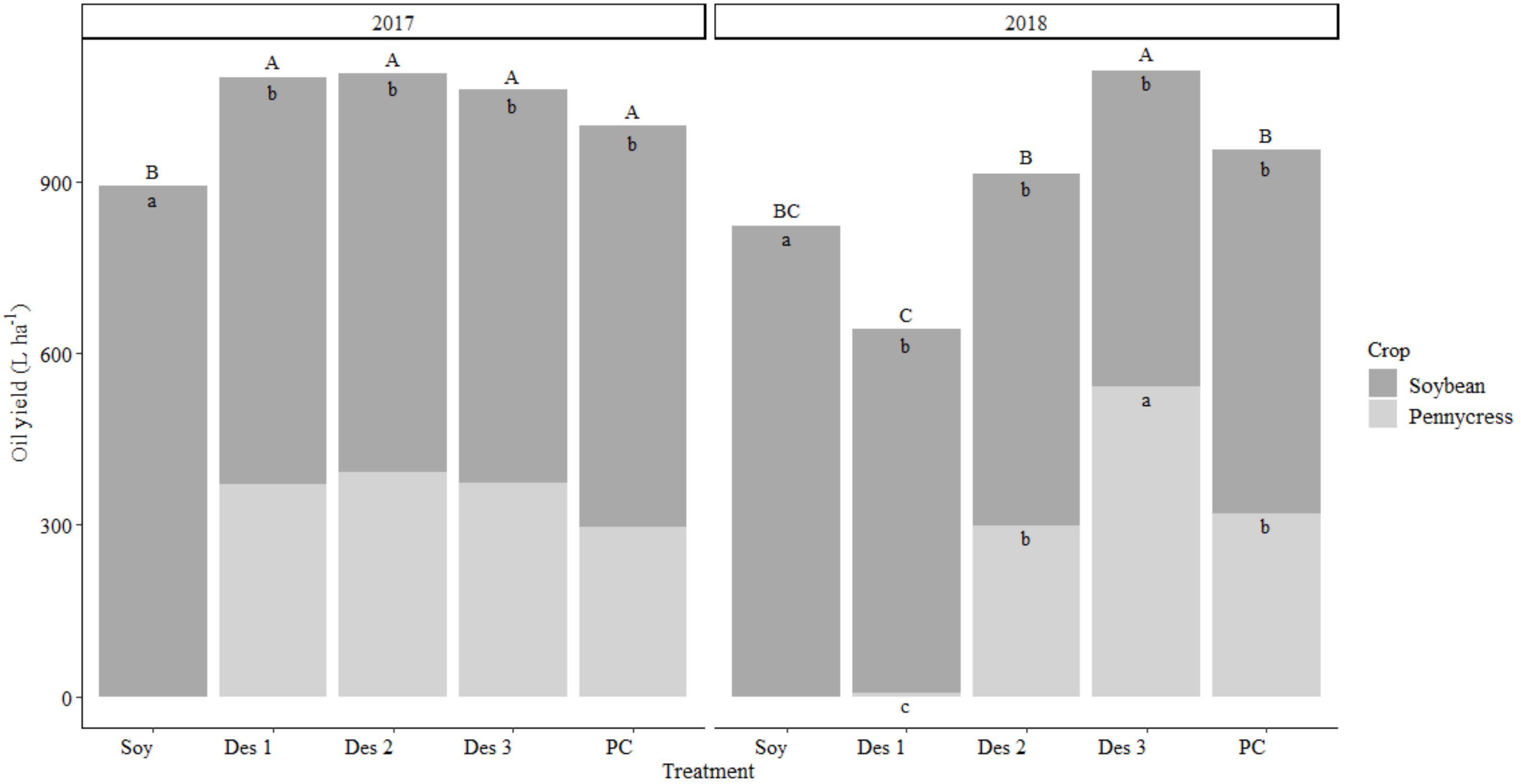

Figure 3-4. Calculated oil yield of pennycress and soybean in Experiment 2 over the 2017 and 2018 harvest seasons. Treatments are the mono-cropped control soybean (Soy), pennycress desiccation date (Des 1-3), and the undesiccated pennycress control (PC). Mean values sharing the same lowercase letter within a year and crop type and bars of aggregate seed yield that share capital letters within a year are not significantly different from each other. Mean values that lack lowercase letters within a year and crop type were not significantly different from each other. All mean separation values are based on Tukey's HSD ($P < 0.05$).

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