OPTIMIZING WINTER CAMELINA PRODUCTION AS A COVER CROP IN NORTH

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**Title**

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CROP IN NORTH DAKOTA

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The Supervisory Committee certifies that this *disquisition* complies with North Dakota

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# ABSTRACT

Winter camelina [*Camelina sativa* (L.) Crantz] has gained particular interested from producers and researchers in the northern Great Plains and local production information is critical. Without a correct sowing date plant stand establishment can be challenging. Morphological differences between winter- and summer-biotypes of camelina can allow producers and researchers to distinguish the two biotypes. Visible and non-visible seed differences can offer effective means to distinguish the two biotypes. Seed quality of camelina is crucial. Sowing in September until the first week of October had similar seed yield. Morphological differences in the upper most developed leaves of camelina seedling can be used to distinguish the two biotypes. Field grown samples of camelina can be analyzed to determine if the seed is winter or summer biotype or a mix of both and we developed a near infrared spectroscopy protocol to determine seed composition of intact camelina seeds.

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# CHAPTER 1. GENERAL INTRODUCTION

Camelina [*Camelina sativa* (L.) Crantz] is an emerging oilseed crop high in omega-3 fatty acids (Belayneh et al., 2015). Camelina has two distinctive biotypes, summer annual and winter annual, with the latter requiring a distinct vernalization period to enter the reproductive phase (Anderson et al., 2018; Chao et al., 2019). Most research and production has used summer annual biotypes in North Dakota and other production regions (Berti et al., 2016). Low seed yield has been a deterrent for the development and adaptation of this crop as an alternative oilseed crop, and currently, camelina is not economically competitive with other cash crops grown in the northern Great Plains (Gesch et al., 2014; Ott et al., 2019). Winter camelina has the potential to serve as a dual-purpose crop; first serving as a winter-hardy cover crop to reduce nitrate leaching (Peterson et al., 2019), soil erosion (Berti et al., 2017b), and excess soil moisture in the spring (Gesch and Johnson, 2015); and then transitioning into an oilseed crop solo or in a double- or relay-cropping system with soybean [*Glycine max* (L.) Merr.] or other short-season crops (Gesch and Archer, 2013).

Timing of winter camelina sowing in the fall is important for stand establishment and winter survival. While winter camelina plants usually survive North Dakota winters, plants may not survive if they are established in late summer or are in early developmental stages at the onset of winter. In addition, the amount of snow cover and temperature fluctuations experienced in the winter can influence overwintering ability of plants. No published research on sowing date of winter camelina is available for producers in North Dakota particularly in the Red River Valley.

In the summer of 2017, several seed companies began to offer camelina seed to meet producers' growing interest in using winter camelina as a cover crop in their crop rotations.

Regrettably, all the seed that was offered by the seed companies was the summer-biotype, which is not able to survive the winter of North Dakota. Due to this, it is imperative that producers are assured that the seed that they will be sowing is of the correct biotype (M.T. Berti, personal communication, 2019).

Having a method that is time and cost effective to identify seed properties such as oil and protein concentration and fatty acid profile is important for any further research to improve camelina seed quality properties. Previous research has developed near-infrared spectroscopy (NIRS) methods to determine many important seed properties in a wide variety of brassica oilseed crops (Singh et al., 2013; Oblath et al., 2016). However, the calibration developed by Oblath et al. (2016) only included one winter- and one summer-biotype of camelina.

To provide researchers working on improvement of camelina with a nondestructive method for determining agronomically important seed properties, development of calibration equations using NIRS are needed.

1.1. Objectives

1. To determine the optimum sowing date of winter camelina to enhance establishment, winter survivability, nitrogen uptake, and seed yield

2. To determine morphological differences between winter- and summer-biotypes of camelina

3. To develop high throughput protocols for determination of field grown winter- and summer-biotypes of camelina using near-infrared spectroscopy

4. To develop non-destructive, high throughput protocols for determining fatty acid profiles and oil and crude protein content in summer- and winter-biotypes of camelina seeds using NIRS

# CHAPTER 2. LITERATURE REVIEW

Camelina [*Camelina sativa* (L.) Crantz] is an oilseed crop in the Brassicaceae family (henceforth brassicas) that is high in omega-3 fatty acids such as linolenic acid (Belayneh et al., 2015). In recent years, there has been tremendous interest in camelina with databases showing 685 publications between 2014 and 2019 with the keyword 'camelina'. Camelina has been confirmed to consist of both summer (spring) and winter-biotypes (Mirek, 1980; Berti et al., 2016; Anderson et al., 2018; Chao et al., 2019). Winter camelina has extensively proven to be particularly winter hardy in the northern Great Plains (Gesch and Cermak, 2011; Gesch et al., 2018; Walia et al., 2018; Peterson et al., 2019). To help farmers to adopt winter camelina as either a winter-hardy cover crop or a cash crop, there must be information available that is applicable to local growing conditions in the northern Great Plains.

## 2.1. Camelina in Cropping Systems

The short-growing season summer- and winter- make camelina easily adapted into various regions and diverse production systems, while proving several ecosystem services including: soil cover, nutrient retention, weed suppression, pollinator habitat, and habitat for other species that can be provided by camelina are currently not appreciated to their full value (Blackshaw et at., 2011; Gesch and Cermak, 2011; Schillinger et al., 2012; Gesch, 2014; Eberle et al., 2015; Berti et al., 2016, 2017b;Jiang and Caldwell 2016; Thom et al., 2016; Gesch et al., 2018; Walia et al., 2018; Hoerning et al., 2019; Ott et al., 2019; Peterson et al., 2019; Stolarski et al., 2019; Zhang and Auer, 2019; Sher and Primack, 2020).

The global human population in 2019 was 7.7 billion, and in 2050, it is expected to reach 9.7 billion (UNDESA, 2019; Sher and Primack, 2020).  This increase of 2 billion people along with the development and industrialization of nations will put large strains on food and energy

production systems worldwide as well as renewable and non-renewable resources (Sher and Primack, 2020). Using sustainable management approaches such as cover crops and dual- and relay-cropping systems will create a more sustainable food and energy production systems while increasing overall production without expanding the land needed for it (Gesch and Archer, 2013; Gesch and Johnson, 2015; Berti et al., 2017a; Johnson et al., 2017; Ott et al., 2019). Relay-cropping, as defined by Heaton et al. (2013), is a sowing of a second crop before the harvest of the first crop so the two crops overlap in part of their life cycles. In the northern Great Plains, the winter hardiness and relatively short life cycle of winter camelina make it well adapted as a cover crop in double- and/or relay-cropping systems (Gesch and Cermak, 2011; Gesch and Archer, 2013; Gesch et al., 2014, Berti et al., 2017a; Gesch et al., 2018; Ott et al., 2019). In order to be economically competitive with other regional crops produced in the northern Great Plains, winter camelina must be grown in a double- or relay-cropping system (Gesch et al., 2014; Ott et al., 2019).  Winter camelina reduces soil nitrate leaching and soil erosion through the winter and spring while maturing early enough to fit a second crop in as double or relay crop (Gesch et al., 2014; Peterson et al., 2019).

Cultivation of summer camelina biotypes has occurred on a limited acreage in the western United States and Canada (Gugel and Falk, 2006; Blackshaw et al., 2011; Schillinger et al., 2012; Aiken et al., 2015; Berti et al., 2016). Most research in North Dakota and elsewhere in the United States and globally has focused on the summer-biotype (Berti et al., 2016). New research on winter camelina biotypes including molecular markers for distinguishing lines as either summer- or winter-biotypes provide additional options for manipulating flowering time and phenotyping of recombinant inbreed lines (Anderson et al., 2018; Chao et al., 2019; Horvath et al., 2019).

2.2. Camelina Fertility and Salinity Tolerance

Camelina has been shown to require lower amounts of nitrogen fertilizer compared to many crops to obtain maximum seed yield, but specific nitrogen fertilizer needs are highly dependent on location, soil type, and precipitation (Wysocki et al., 2013). While camelina has often been touted as a lower nitrogen requiring crop, research has demonstrated a similar nitrogen requirement as several other species including brown mustard [*Brassica juncea* (L.) Czern] and canola (*B. napus* L.). In the Canadian Prairie Provinces, camelina has been shown to respond similarly to brown mustard with maximum seed yield achieved at 170 kg N ha$^{-1}$ (Malhi et al., 2014). Current N fertilizer recommendations in North Dakota for canola are 167 kg N ha$^{-1}$ for the eastern side of the state and 133 kg N ha$^{-1}$ on the western side (Franzen, 2018), which is similar to results published for camelina by Malhi et al. (2014). Winter camelina is an attractive alternative crop when it is utilized in a relay crop system with soybean (Gesch and Archer, 2013; Ott et al., 2019). While increasing N fertilizer does result in an increased yield, it can lead to increased incidence of downy mildew (*Hyaloperonospora camelinae*) infection (Jiang and Caldwell, 2016) and increased lodging in camelina (Solis et al., 2013). Nitrogen fertilization rate has also been shown to be negatively correlated with glucosinolates content in camelina seed, which could be an added benefit for end users of the seed meal, but probably the increased cost of fertilizer would be greater than the value of glucosinolate reduction (Jiang et al., 2016).

Thousand-seed weight is an important yield component of all crops and has been shown to increase by increasing N rates (Jiang and Caldwell, 2016). While achieving maximum seed yield and 1000-seed weight with additional N can be beneficial, special attention must be given to the potential negative effects of N fertilization such as increased production costs and likely increased nitrate leaching and nitrous oxide emissions (Basche et al., 2014; Turkeltaub et al.,

2016). Residual N fertilizers applied but not taken up by the crop could negatively affect the environment resulting in increased acidification potential, human toxicity and indirectly global warming (Berti et al., 2017b).

Interactions or main effects of N, P, and S fertilization rates on 1000-seed weight were not observed (Solis et al., 2013). Camelina in South Central Chile exhibited no significant response to S fertilization (Solis et al., 2013) unlike other brassica species such as canola that exhibits strong responses to S fertilizer (Franzen, 2018). Camelina has a salinity threshold of 8.0 $dSm^{-1}$ and a 25% germination decline at 35.3 $dSm^{-1}$ making it tolerant to salinity during germination, and therefore, making it more attractive than other alternative oilseed crops such as field pennycress (*Thlaspi arvense* L.), *Echium plantagineum* L., *Cuphea viscosissima x Cuphea lanceolata* W.T. Aiton, and *Calendula officinalis* L. (Matthees et al., 2018). Having a salinity tolerant cover crop or crop would be of great value to producers especially in the Red River Valley, USA as there are large areas of salinity-affected soil, including affected areas present along field margins or low spots (Skarie et al., 1986).

2.3. Biotic Interactions with Camelina

In recent years, there has been increased concern globally over the decline in pollinator populations worldwide (National Research Council, 2007). New oilseed crops can help provide season-long pollen production (Thom et al., 2018). One hectare of winter camelina could support between two and four colonies of European honey bees (*Apis mellifera* L.) at a crucial time in the spring when other pollen and nectar sources are limited (Thom et al., 2018). Field pennycress, winter camelina, and winter canola all enter reproduction and begin to flower between late May and early June in central Minnesota, not only providing pollen to honey bee colonies that are returning from winter apiaries but also to resident insects emerging from winter hibernation

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(Eberle et al., 2015). Winter camelina compared with field pennycress had higher overall visitation rates by insects (Eberle et al., 2015; Thom et al., 2016). Honey bees have also been reported to only visit camelina but not field pennycress in a study conducted in southern Germany (Groeneveld and Klein, 2014). In Minnesota and South Dakota, both winter camelina and field pennycress were both visited during pollination by honey bees, but winter camelina had the most potential as a nectar source for pollinating insects (Eberle et al., 2015). Camelina has also shown resistance to insect pests while providing a food source for beneficial insects (Gugel and Falk, 2006; Thom et al., 2018; Eberle et al., 2015). Metspalu et al. (2014) demonstrated that camelina is not a host for cruciferous flea beetles (*Chrysomelidae: Alticinae*) including *Chaetoncema concinna*, *Phyllotreta undulata, P. nemorum, P. vittata, and P. nigripes,* however, the exact reason of lack of infestation is not known. This is an added benefit for producers who are looking for a relatively low input alternative crop compared with canola, which is very susceptible to flea beetles (Kandel et al., 2019). Winter camelina (cv. Joelle) also has been demonstrated to show no reproduction of two soybean cyst nematode (*Heterodera glycines* Ichinohe, SCN) populations under controlled conditions (Acharya et al., 2019), which has become a major pest in soybean producing states in the U.S. (Tylka and Marett, 2017). Weed species including field pennycress which is also being investigated, as another alternative winter annual oilseed crop, is also a host for SCN (Johnson et al., 2008; Poromarto et al., 2015). It is also important to indicate that camelina as well as other cover crops and weeds in the brassicaceae family are hosts for club root (*Plasmodiophora brassicacea* Woronin), a major soil borne disease of canola (Seguin-Swartz et al., 2009; Chapara, 2019).

2.4. Winter Camelina as a Winter Hardy Cover Crop

Traditionally, the only cover crop that consistently survives the northern Great Plains winters is winter rye (*Secale cereale* L.) (Appelgate et al., 2017; Thomas et al., 2017; Peterson et al., 2019). A winter-hardy broadleaf cover crop such as winter camelina can be a valuable resource for producers looking to fill voids that winter rye cannot provide as a cover crop in their crop rotations. Winter rye spring growth can cause soil water depletion affecting corn emergence and establishment or it can delay corn sowing until adequate soil moisture is present once again after termination of the winter rye, both of which can be more pronounced when precipitation is below the long-term average (Krueger et al., 2011). The shorter the number of days between termination of winter rye and sowing of corn resulted in increased seedling disease (Acharya et al., 2017). Seedling pathogens of *Fusarium graminearum, F. oxysporum, Pythium sylvaticum*, and *P. torulosum* have been shown to increase in number on the root system of winter rye that was herbicide-terminated before corn planting, demonstrating a wide potential for increased disease pressure in corn (Bakker et al., 2016). Additionally, N accumulation and assimilation in winter rye biomass resulted in less than sufficient amounts of N to supply subsequent dryland spring wheat (*Triticum aestivum* L.) crops in Alberta, Canada, and thus leading to a 38% reduction of yield compared with no fall-sown cover crop (Thomas et al., 2017). Winter rye and camelina intersown into standing soybean resulted in the lowest spring residual soil $NO_3$-N levels (Peterson et al., 2019). Intersown winter camelina and winter rye also reduced soil $NO_3$-N significantly compared to summer annual cover crops and a check with no cover crop (Peterson, et al., 2019) Subsequently intersown winter rye decreased spring wheat yields compared with intersown: winter camelina, summer annual cover crops and a check with no cover crop

(Peterson et al., 2019). Further research is needed to provide producers with the necessary information to efficiently use winter camelina on their farming operations in different scenarios.

2.5. Camelina Morphology, Growth, and Development

Morphological differences between winter- and summer-biotypes of camelina have been previously noted by several researchers, but no research had been conducted to provide accurate descriptions of both biotypes. A phenological growth stage scale of camelina was developed by Martinelli and Galasso (2011) using the extended BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) scale. Martinelli and Galasso (2011) described ten different growth stages of camelina using the BBCH two- and three-digit coding system. The coding system along with accompanying drawings provide a valuable tool for researchers in production and physiological studies of camelina.

Camelina has a relatively short life cycle compared with other crops grown in the western United States and Canada. Studies focusing on summer camelina have generally used a base temperature of 5°C (Gesch, 2014; Sintim et al., 2016; Zanetti et al., 2017). In West central Minnesota, ten summer camelina cultivars were evaluated across nine total sowing dates and three years. In this study, days from planting to 50% flowering ranged from 36 days to 59 days (Gesch, 2014). The less days between planting and 50% flowering was due to increasing growth temperatures, corresponding to a range of 469 to 578°C growing degree days (GDD: 5°C base temperature). Gesch (2014) reported the range of days from sowing to harvest was 75 to 100, corresponding to a range in GDDs of 1101 to 1216°C GDDs. While there were significant differences between treatments for GDDs and days to 50% flowering, there was no significant difference in either accumulated GDDs or days to harvest (Gesch, 2014). Sintim et al. (2016a) reported significant differences in the number of days to harvest but not in GDDs to harvest in

Wyoming. Reports also show that early sowing dates from April to May result in higher seed yield (Gesch, 2014; Sintim et al., 2016a). Delaying sowing of summer camelina until later in the growing season results in uneven emergence and supplemental irrigation may be required to achieve the stands required for maximum seed yield (Dobre et al., 2014).

Several studies focusing on winter camelina have used a GDD base temperature of 4°C (Gesch and Cermak, 2011; Gesch et al., 2018; Walia et al., 2018). Since a lower base temperature was used and longer period between fall sowing and summer harvest for winter-biotypes, the GDD accumulation to 50% flowering is considerably higher than research done with summer-biotypes. Winter camelina cultivars grown in 2013-2014, 2014-2015, and 2015-2016 growing seasons required different GDDs to reach 50% flowering. Gesch and Cermak (2011) did not determine significant differences in GDDs to 50% flowering between cultivars BSX-WG1 and Joelle across growing season 2007-2008 and 2008-2009. Higher temperatures in both the fall and spring were shown to increase the GDD accumulated but the days to 50% flowering was late May to early June, regardless of accumulated GDDs (Gesch and Cermak, 2011).

Camelina root system is small and shallow having 82% of its roots located within 0.3-m of the soil surface at harvest (Gesch and Johnson, 2015). In winter camelina-soybean double and relay cropping systems, the average of in-season water usage was 26 and 50mm more than monocrop soybean in 2010 and 2011, respectively (Gesch and Johnson, 2015). Summer camelina is a low water usage crop as irrigation amounts ranging from 330, 314, 281, and 295 mm corresponding to soil water depletion levels of 45, 50, 60, and 75% did not differ in seed yield (Hunsaker et al., 2011). Camelina surpassed canola, *Brassica carinata* L., and *B. juncea* in productivity, however, was not different, under warmer and drier growing conditions which

could possibly be explained by camelina's shorter plant structure, higher harvest index, more efficient desaturation pathway, converting oleic fatty acid to linoleic and linolenic fatty acids, and its resistance to flea beetles (Aiken et al., 2015; Enjalbert et al., 2013). Seed size of camelina is quite small, ranging from 0.8 to 1.8 g per thousand seeds (Berti et al., 2016), but has been demonstrated to have faster emergence than canola, and brown mustard at low temperatures, which can allow for earlier planting under field conditions (Enjalbert et al., 2013). Under controlled environments, germination rates reached 100% except at 32°C when tested at 4, 10, 16, 21, 27, and 32°C (Russo et al., 2010). At the 4°C, maximum germination was reached at nine days, therefore demonstrating that camelina seed can germinate under low-temperature conditions (Russo et al., 2010). Base temperature for 50% germination of five summer camelina cultivars using linear regression was calculated to be -0.7°C in a controlled incubation study, demonstrating that camelina can be sown relatively early in the spring but often can be delayed because of lack of field access (Allen et al., 2014). Sowing of camelina is done very shallow due to its small seed size, however, less than adequate stands for high seed yield and weed competition may be achieved due to variable soil moisture (McVay and Khan, 2011; Gesch et al., 2017). Allen et al. (2014) demonstrated that emergence was 11% quicker at a 3-mm sowing depth compared with a 6-mm sowing depth under controlled incubator conditions. Using a hoe-drill in camelina field production resulted in better seedling emergence and stand development, but no-till drills also resulted in adequate stands for canola, camelina, and brown mustard (Aiken et al., 2015).

2.6. Seed Composition

While camelina is known for its high concentration of omega-3 fatty acids, its total oil yield is low when compared with other oilseed crops that can be used to produce hydro-treated

renewable jet fuel (HRJ) (Gesch et al., 2015). Researchers determined that camelina and white mustard (*Sinapis alba* L.) had the lowest oil yield per hectare compared with canola, *Brassica rapa* L., *B. juncea* and, *B. carinata* in West central Minnesota (Gesch et al., 2015). Camelina has been shown to have a good combination of seed yield and seed oil yield to serve as feedstock for biodiesel production as a substitute for canola (Blackshaw et al., 2011; Ciubota-Rosie et al., 2013; Singh et al., 2014 Yang et al., 2016). Raw camelina oil has been demonstrated to contain high levels of natural antioxidants such as vitamin E (Ibrahim and el Habbasha, 2015). Camelina meal can also be utilized for a number of products including bio-herbicides, soil fungicides, adhesives, bio-oils, and various animal feed (Berti et al., 2016). Harvest timing has been shown to influence the composition of various fatty acids in the seed including increases in linolenic acid formed from desaturation of linoleic acid, which is important to monitor for end uses with different quality requirements, especially those looking for high omega-3 fatty acid sources (Walia et al., 2018). Time of harvest can also influence overall seed yield as seed shattering in camelina can be a major problem (Sintim et al., 2016b). Earlier harvest with the aid of swathing or desiccants of camelina has shown to reduce shattering losses (Sintim et al., 2016b; Walia et al., 2018).

Standard determination methods of seed crude protein and oil concentration and fatty acid profile are time-consuming processes that result in the destruction of the seed in procedures from the AOAC Official methods Analysis 920.39 and 988.05 (1990) and Vick et al. (2004). Contrarily, broad near-infrared spectroscopy (NIRS) scanning of seed results in fast, cost-efficient, and non-destructive analysis for various constituents (Singh et al., 2013; Oblath et al., 2016; Zhang et al., 2017). Previous research has been conducted to develop a NIRS calibration to predict seed quality characteristics of various brassica species. Oblath et al. (2016) developed a

calibration for the constituents of seed moisture, total oil, 12 fatty acids, N, glucosinolates, and chlorophyll content, and included in the calibration were six different species including *Brassica napus*, *B. carinata*, *B. juncea, B. rapa*, *Sinapis alba,* and camelina (Oblath et al., 2016). From these six species, a total of 367 samples were scanned with NIRS and prediction equations were developed with reference values obtained from wet chemistry; the resulting calibrations that were developed produced good results, but they only included two camelina samples, one winter- and one summer-biotype (Oblath et al., 2016). Near-infrared spectroscopy calibrations have also been developed to predict oil content of camelina seed, and the research obtained a strong prediction equation with a $r^2$ of 0.94 based on a calibration using 200 samples (Zhang et al., 2017). High linolenic acid levels within the seed have been shown to be correlated with high levels of linolenic acid in leaf-membranes resulting in superior drought and cold tolerance of camelina (Enjalbert et al., 2013). Having a non-destructive and rapid method to determine linolenic acid and other seed properties will be of tremendous benefit for camelina breeders. Genetics and the environment where camelina are produced influence fatty acid profile and yield allowing NIRS calibrations to be used for producers and researchers (Obour et al., 2017; Zhang et al., 2017).

# CHAPTER 3. WINTER CAMELINA SOWING DATES

## 3.1. Abstract

Camelina [*Camelina sativa* (L.) Crantz], is a member of *Brassicaceae* family, which has the potential to serve as a low-input crop in the northern Great Plains especially in a double- or relay- cropping system to provide oil for both advanced biofuels but also for human consumption. Winter annual camelina can result in economic and environmental benefits for the northern Great Plains, however little is known about their agronomic potential in the region. A preliminary study was established in the summer and fall of 2017 and in the summer and fall 2018 to determine optimum sowing dates for achieving the highest winter camelina seed yield as well as several ecosystem services. Sowing dates ranged from the end of June to mid- October over the two growing seasons the experiment was conducted. The experimental design was a randomized complete block (RCBD) with four replicates at each location. Fall stand counts ranged from 17 to 279 plants $m^2$ being less than the sowing rate of 700 pure live seeds per $m^2$, with more plants at later fall sowing dates. Spring stand counts ranged from 7 to 84 plants per $m^2$, with higher stand counts at sowing dates sown from the beginning to mid-September. Across sowing dates that survived the winter, seed yield ranged from 99 to 1317 kg $ha^{-1}$. Results from these three environments indicate that when sown in September and even into October, plants can successfully survive the winter and produce a harvestable crop in the subsequent growing season in the northern Great Plains.

## 3.2. Introduction

Camelina sowing date experiments were conducted at Fargo, ND in the 2017 to 2018 growing season (46.90025 N and -96.80507 W, elevation 273 m) at the United States Department of Agriculture – Agriculture Research Service site and in the 2018 to 2019 growing

season, at two North Dakota State University (NDSU) research sites in Fargo, ND (46.895433 N and -96.816244 W, elevation 273 m) and Prosper, ND (46.9992 N and -97.114779 W, elevation 277 m). Soils at Fargo consist of the Fargo soil series (fine, smectitic, frigid Typic Epiaquerts), while Prosper soils are a complex of Kindred (fine-silty, mixed, superactive, frigid Typic Endoaquolls) and Bearden (fine-silty, mixed, superactive, frigid Aeric Calciaquolls) soil series (Soil Survey Staff, 2005; Soil Survey Staff, 2014; Soil Survey Staff, 2016).

The North Dakota Agriculture Weather Network (NDAWN) system at all three environments recorded daily temperature and rainfall (NDAWN, 2019). Soil samples were taken in Fargo and Prosper in 2018 prior to the first sowing date per replicate. Prior to sowing in the summer of 2017, one set of soil samples was taken on that experimental location. Soil analysis that was performed on 0- to 15-cm depth samples included: pH, organic matter, P, and K (Table 1) (Franzen, 2018). The $NO_3$-N analysis was performed on samples taken both at 0- to 15-cm and 15- to 60-cm depths (Table 1).

Table 1. Initial soil analysis for experimental sites in Fargo and Prosper, ND in the 2017 to 2018 and 2018 to 2019 growing season.

| Sites | pH | OM g kg$^{-1}$ | P mg kg$^{-1}$ | K mg kg$^{-1}$ | NO$_3$-N (0-15-cm depth) kg ha$^{-1}$ | (15-60-cm depth) kg ha$^{-1}$ |
|---|---|---|---|---|---|---|
| 2017 - 2018 | | | | | | |
| Fargo | 7.4 | 84.0 | 46.0 | 485.0 | 219.5 | 490.6 |
| 2018 - 2019 | | | | | | |
| Fargo | 7.7 | 75.4 | 15.1 | 307.8 | 116.1 | 181.0 |
| Prosper | 7.8 | 43.2 | 27.0 | 310.5 | 52.1 | 110.0 |

OM = Organic matter.

At each sowing date in the fall of 2018, soil samples were taken at 0- to 15-cm depth and placed into a metal tin to determine soil gravimetric water content in each experimental unit to be sown. Soon after, weight of the wet soil samples and tins were taken. After initial weights were collected the lids were removed, and the samples were then placed into an oven at a temperature

of 105°C for 24 h to ensure all water was removed from the soil (Black, 1965). After 24 h, the

tins where removed from the dryer, and weighed immediately, the weights were recorded in

order to complete the calculation of gravimetric water content on a dry weight basis.

Additionally, there was no previous crop planted on the experiment site at all locations.

Experiments were conducted using a RCBD with four replicates. Six sowing dates were

conducted in the summer and fall of 2018, however, at Fargo in 2017 five sowing dates were

possible due to excessive rainfall. The five sowing dates in Fargo in 2017 were sown from 30

June to 6 October (Table 2).  In 2018, six sowing dates were sown from 31 July to 17 October in

Fargo (Table 2). In 2018, the six sowing dates were sown from 31 July to 23 October in Prosper

(Table 2).

Table 2. Sowing dates at Fargo and Prosper in 2017 and 2018.

| Location/Year | Sowing dates | | | | | |
|---|---|---|---|---|---|---|
| | Date 1 | Date 2 | Date 3 | Date 4 | Date 5 | Date 6 |
| Fargo 2017 | 30 June | 26 July | - | 24 Aug. | 8 Sept. | 6 Oct. |
| Fargo 2018 | 31 July | 14 Aug. | 6 Sept. | 11 Sept. | 1 Oct. | 17 Oct. |
| Prosper 2018 | 31 July | 14 Aug. | 31 Aug. | 11 Sept. | 1 Oct. | 23 Oct. |

Winter-annual camelina cv. Joelle was used and the sowing rate was 5.6 kg ha $^{-1}$ of pure

live seed (PLS). Germination of 'Joelle' was tested prior to the first sowing date in each year and

was 47% and 80% in 2017 and 2018, respectively. Germination was determined by counting out

a sub sample of 100 seeds of the lot that was determined to serve as seed for this experiment, the

subsample of seed was placed on moist germination paper and after one week the number of

germinated seeds was counted to determine germination of the seed lot.  At a 1000-seed weight

of 0.7 g, approximately 1.1 million viable seeds were sown per kg of seed. Experimental units

were 7.6 m long and 1.2 m wide consisting of eight rows 15 cm apart. Sowing dates ranged from

the end of June to mid-October. Sowing depth for all sowing dates was 6 mm. Pre-plant spraying

of glyphosate (N-(phosphonomethyl) glycine) (1.2 kg active ingredient (a.i.) ha$^{-1}$) was performed at both locations in 2018 prior to the first sowing date. A second pre-sowing application of glyphosate (1.2 kg a.i. ha$^{-1}$) was made on 21 August in Fargo only to experimental units that had not been sown yet. Applications of glyphosate was applied to control emerged weeds at the time of applications.

*3.2.1. Plant Sampling and Soil Analysis*

Shortly before fall growth was expected to cease by predicted hard frost date of 1 November, plant biomass samples were collected by hand cutting 0.19 m$^2$ from the outside two rows in experimental units where it was deemed enough biomass was present. This was done to avoid influencing seed yield in the following season. Fall biomass was collected on 30 October and 31 October in Fargo and Prosper in 2018, respectively. At the same time, fall biomass samples were collected, plant stand counts were determined in 0.19 m$^2$ on the middle-two rows of each experimental units only in the fall of 2018. No fall biomass or stand counts were collected in Fargo in 2017, as it was a preliminary experiment. Biomass samples were dried (40°C for 7 d) to weigh and record dry weight. Dried samples were then ground to 1-mm size particles with a Model 4 cutting mill (Eberbach Corporation, Ann Arbor, MI, USA). Biomass N was determined by running samples with a XDS Near Infrared Rapid Content using a calibration previously developed in our lab for camelina biomass composition. Spring biomass was also collected on the outside two rows from 0.19 m$^2$ in experimental units deemed to have enough biomass prior to bolting prior to BBCH 301 (Martinelli and Galasso, 2010), and samples were collected on 14 May and 7 May in 2018 and 2019, respectively. At the same time, spring biomass samples were collected, plant stand counts were determined in 0.19 m$^2$ on the middle-two rows of each experimental units only in the spring of 2019. As camelina progressed through

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bolting and then into reproductive stages, flowering date was recorded based on visual observations of the whole experimental unit when at least 50% of the plants had at least one open flower. Biomass N accumulation was determined by multiplying aboveground biomass yield by N content in biomass obtained.

When camelina plants in experimental units reached harvest maturity, average overall plant height was determined for each experimental unit by measuring three random plants in each experimental unit. Visual rating of shattered silicles and intensity of powdery mildew were taken in Fargo in 2019 prior to harvest, as a degree of shattering was present and powdery mildew was only experienced in Fargo in 2019. Prior to mechanical bulk harvest of the experimental units, 2 m from the middle two rows were harvested by cutting the plants off at the soil surface and then placing them into cloth bags; these total biomass samples were used for calculation and analysis of the harvest index of the experimental units. While harvesting the area for calculation of harvest index, the number of plants harvested were also noted to determine harvested stand. Calculation of harvest stand and index was done in all three locations. In addition to harvesting 2-m, two individual plants were taken from each experimental unit and placed in separate paper bags to determine number of silicles per plant that were present, the number that had already shattered (septum presence), total number of silicles, number of seeds per silicle, 1000-seed weight, and seed yield per plant in Fargo and Prosper 2019. Harvest index, and seed yield components from the field were dried in dryers at a temperature of 33°C until all had approximately 40 g kg$^{-1}$ of water content. Harvest index samples were taken from the dryers and threshed by hand to separate the seed from the biomass. The seed was cleaned using hand screens; once the seed was clean, it was weighed as well as the biomass for calculation of harvest index. Plant samples were taken from the dryers, and the number of silicles present and already

shattered (septum remaining after seed shattering) were recorded. Twenty random silicles per plant were threshed individually to calculate the seeds per silicle. The remaining silicles were then threshed together, and the seed was cleaned using hand screens. Once the seeds were cleaned and weighed seed yield per plant and harvest index of each individual plant were calculated. Thousand-seed weight was determined by selecting a subsample of exactly 100 seeds. This subsample was then weighed in a precision scale, and the resultant weight was multiplied by a factor of 10 in order to obtain 1000-seed weight. Seed samples from these plant samples were returned to the primary seed yield samples after analysis.

Following collection of biomass samples, the mechanical harvest of the whole experimental units was completed using a Hege 125B plot combine (Hans-Ulrich Hege Company, Waldenberg, Germany). During harvest, combine header height was maintained so that all silicles were collected and threshed. Harvest dates were 13 July 2018, 24 July 2019, and 22 July 2019 for experiments sown in Fargo in 2017 and in Fargo and Prosper in 2018, respectively. Seed yield samples from the field were dried in dryers at a temperature of 33°C until all had approximately 40 g kg$^{-1}$ of water content. Bulk yield samples harvested with Hege 125B were cleaned using a Clipper Office Tester Model (Clipper, A.T. Ferrell Company, Bluffton, IN) and then transferred into cloth bags.

The harvested seed yield was analyzed for quality components such as crude protein, N, and total fat content along with a fatty acid profile of the oil by scanning samples with a XDS Near Infrared Rapid Content Analyzer (Foss, Copenhagen, Denmark) using equations developed previously in our laboratory. In Fargo, in 2019 following harvest, the number of shattered silicles present on the ground were counted in a random 0.093 m$^2$ area to calculate the approximate amount of seeds lost from shattering and suspected bird damage prior to harvest. Seed water

content was obtained from the XDS Near Infrared Rapid Content Analyzer and was used to correct seed yield at a seed water content of 85 g kg$^{-1}$.

Fall soil samples in 2018 were analyzed for $NO_3$-N at depths of 0- to 15-cm and 15- to 60-cm in all experimental units regardless if there were plants present or not. In the following spring, soil samples were only taken in experimental units with plants that had survived the winter and then analyzed for $NO_3$-N using trans-nitration of salicylic acid methods (Vendrell and Zupancic, 1990).

*3.2.2. Statistical Analysis*

Statistical analysis was conducted using SAS software, procedure MIXED (S.A.S. Institute, 2014) using standard procedures for a randomized complete-block design. Each location-year combination was considered an "environment" and random effect, while sowing date was considered a fixed effect.

Analysis of variance was conducted within and across two environments. If the error mean squares of the environments of the 2018 to 2019 growing season locations were homogenous, then a combined analysis was conducted. A mean separation test was performed using the *F*-protected LSD at $P \leq 0.05$ level of significance for each measured trait.

3.3. Results and Discussion

*3.3.1. Rainfall and Temperature*

The Fargo environment was very dry in both summer and fall of 2017 and the following spring (Fig. 1). With the total amount of rainfall being 275 mm less than the 30-yr average. June and July were very dry months, experiencing rainfall amounts of 42 and 48 mm below the 30-yr average monthly rainfall, respectively (Table 3). This was very critical for the first two sowing dates, while also affecting later sowing dates because of decreased soil water availability.

October was also well below the 30-yr average rainfall by 34 mm. This season-long moisture deficiency hindered adequate stand establishment and vegetative growth in the fall. The spring of 2018 also was below the 30-yr average rainfall for the months of April and May by 29 and 28 mm, respectively. While in June and July, rainfall was above the average by 24 and 10 mm, respectively. May and June also had average maximum temperatures of 25 and 27°C, which were 4.7 and 1.9°C above the 30-yr average, respectively (Fig. 1). Average minimum temperatures were also higher than the 30-yr average by 3.3 and 2.8°C in May and June, respectively. Growing degree day accumulation to both 50% flowering and harvest were affected by the increased temperature in May and June (Table 4).

Table 3. Total monthly rainfall, temperature, and departure from normal for the environment at Fargo in 2017 and 2018.

| Environment | Month | Rainfall | | Temperature | | | |
| | | Total | ±Normal[a] | Max. | ±Normal[a] | Min. | ±Normal[a] |
|---|---|---|---|---|---|---|---|
| | | ----------mm-------- | | ----------------------°C---------------------- | | | |
| Fargo | April | 25.1 | -9.4 | 14.1 | 0.8 | 1.1 | 0.7 |
| 2017 | May | 26.5 | -44.9 | 20.6 | -0.1 | 7.4 | 0.2 |
| | June | 57.5 | -41.6 | 26.4 | 1.1 | 13.2 | 0.4 |
| | July | 22.6 | -48.3 | 29.0 | 0.9 | 15.6 | 0.2 |
| | Aug | 58.2 | -6.8 | 25.4 | -1.9 | 13.2 | -0.9 |
| | Sept | 69.9 | 4.6 | 22.4 | 0.9 | 10.5 | 2.0 |
| | Oct | 20.3 | -34.3 | 15.0 | 1.6 | 2.4 | 0.6 |
| | Nov | - | - | 2.6 | -0.4 | -6.7 | -0.2 |
| | | | | | | | |
| Fargo | April | 5.8 | -28.7 | 7.2 | -6.0 | -3.9 | -4.3 |
| 2018 | May | 43.6 | -27.7 | 25.4 | 4.7 | 10.5 | 3.3 |
| | June | 123.2 | 24.1 | 27.2 | 1.9 | 15.6 | 2.8 |
| | July | 80.9 | 10.0 | 27.8 | -0.2 | 16.0 | 0.6 |

Weather data obtained from: https://ndawn.ndsu.nodak.edu/weather-data-monthly.html
[a]Based on 1981-2010 long-term averages

Table 4. Growing degree days (GDD) for Fargo, ND in 2017 and 2018.

| Sowing date | Sowing to end of fall GDD[a] | GDD 1 April to 50% flowering | GDD 1 April to harvest | Total GDD |
|---|---|---|---|---|
| | ------------------------------------°C d ------------------------------------ | | | |
| Date 1 | 1603 | - | - | - |
| Date 2 | 1140 | - | - | - |
| Date 3[b] | - | - | - | - |
| Date 4 | 673 | 274 | 1273 | 1946 |
| Date 5 | 453 | 304 | 1273 | 1726 |
| Date 6 | 132 | 443 | 1273 | 1405 |

[a] GDD base temperature = 4°C
[b] Sowing date 3 was skipped due to excessive moisture.
Last date in fall was determined by hard freeze below -7°C which occurred on October 31.
Spring and total GDDs were only calculated for experimental units that survived the winter.
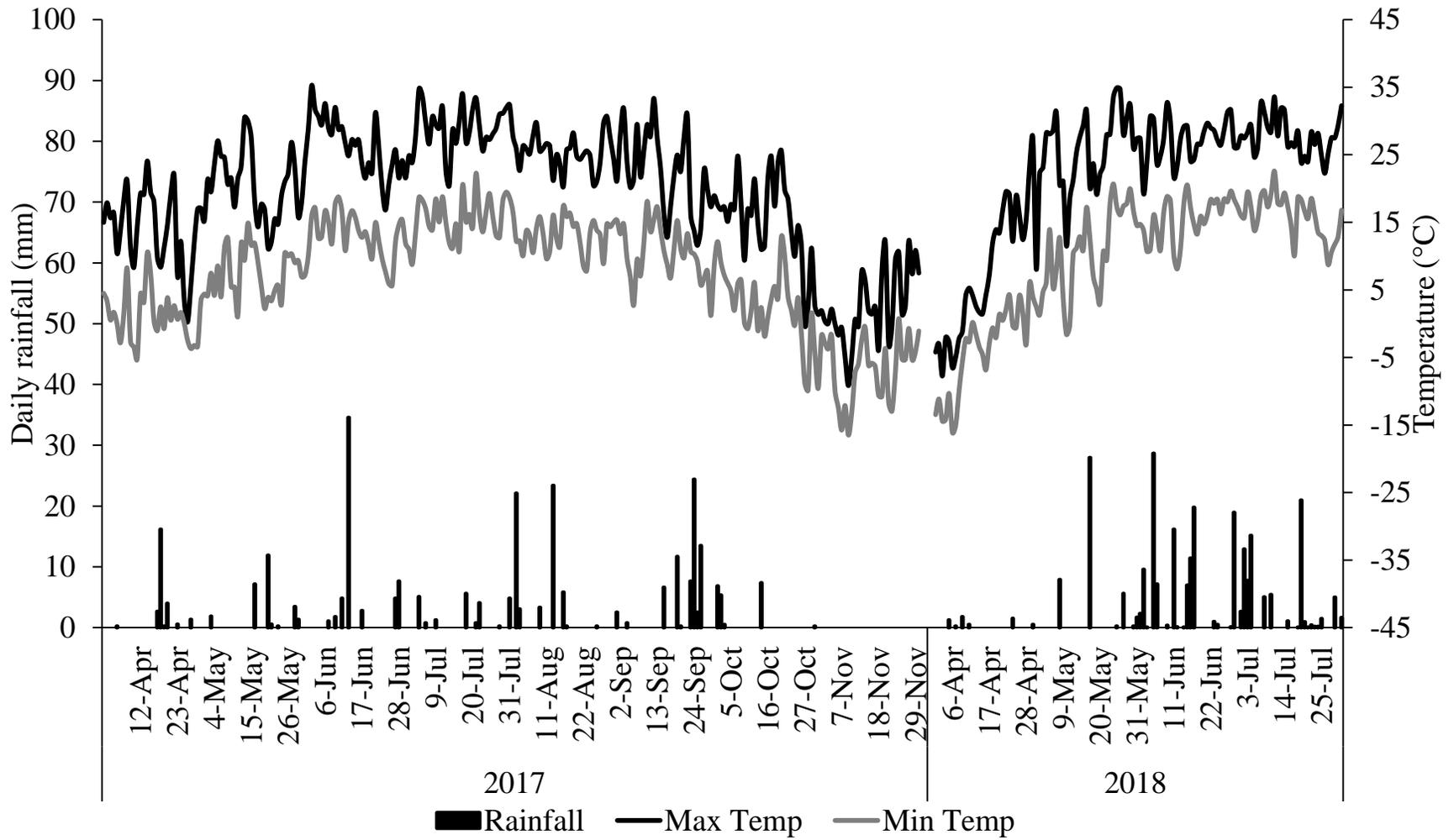
Fig. 1. Daily total rainfall and maximum and minimum temperature for Fargo in 2017 to 2018.

In 2018, in Fargo, the summer and fall growing season was marked by considerably more rainfall than in the fall of 2017 (Fig. 1, Fig, 2). July and August had 10.0 and 35.9 mm more rainfall, respectively than the 30-yr average while September was 0.9 mm below the 30-yr average and November was 3.5 mm above the 30-yr average (Table 5). In the following spring, the difference in rainfall compared with the previous fall was 10.1, 1.7, and 16.3 mm below the 30-yr average being observed for the months of April, May, and June respectively. In July, rainfall precipitation was 50.4 mm above the 30-yr average. Average temperatures in April and May were below the 30-yr average resulting in decreased GDD accumulation to 50% flowering (Table 6). While in the months of June and July the average temperature was above the 30-yr average and resulted in increased GDD accumulation to harvest (Table 6).

Table 5. Total monthly rainfall, temperature, and departure from normal for the environment at Fargo in 2018 and 2019.

| Environment | Month | Rainfall | | Temperature | | | |
|---|---|---|---|---|---|---|---|
| | | Total | ±Normal[a] | Max. | ±Normal[a] | Min. | ±Normal[a] |
| | | ----------mm--------- | | ----------------------°C----------------------- | | | |
| Fargo | April | 5.8 | -28.7 | 7.2 | -6.0 | -3.9 | -4.3 |
| 2018 | May | 43.6 | -27.7 | 25.4 | 4.7 | 10.5 | 3.3 |
| | June | 123.2 | 24.1 | 27.2 | 1.9 | 15.6 | 2.8 |
| | July | 80.9 | 10.0 | 27.8 | -0.2 | 16.0 | 0.6 |
| | Aug | 100.9 | 35.9 | 27.0 | -0.3 | 14.6 | 0.5 |
| | Sept | 64.3 | -0.9 | 20.9 | -0.7 | 9.0 | 0.4 |
| | Oct | 58.1 | 3.5 | 9.3 | -4.1 | 0.0 | -1.7 |
| | Nov | - | - | -1.7 | -4.7 | -8.9 | -2.4 |
| | | | | | | | |
| Fargo | April | 24.4 | -10.1 | 10.7 | -2.5 | 1.6 | 1.2 |
| 2019 | May | 69.6 | -1.7 | 17.6 | -3.2 | 5.3 | -1.9 |
| | June | 82.8 | -16.3 | 25.6 | 0.4 | 13.7 | 1.0 |
| | July | 121.3 | 50.4 | 27.9 | -0.2 | 17.1 | 1.7 |

Weather data obtained from: https://ndawn.ndsu.nodak.edu/weather-data-monthly.html
[a]Based on 1981-2010 long-term averages

Table 6. Growing degree days (GDD) for Fargo, ND in 2018 and 2019.

| Sowing date | Fall GDD[a] | GDD 1 April to 50% flowering | GDD 1 April to Harvest | Total GDD |
|---|---|---|---|---|
| ---------------------------------°C d --------------------------------- | | | | |
| Date 1 | 924 | - | - | - |
| Date 2 | 663 | 416 | 1253 | 1916 |
| Date 3 | 311 | 290 | 1253 | 1564 |
| Date 4 | 239 | 321 | 1253 | 1492 |
| Date 5 | 55 | 377 | 1253 | 1308 |
| Date 6 | 38 | - | - | - |

[a] GDD base temperature = 4°C

Last date in in fall was determined by hard freeze below -7°C which occurred on November 7.
Spring and total GDDs were only calculated for experimental units that survived the winter.
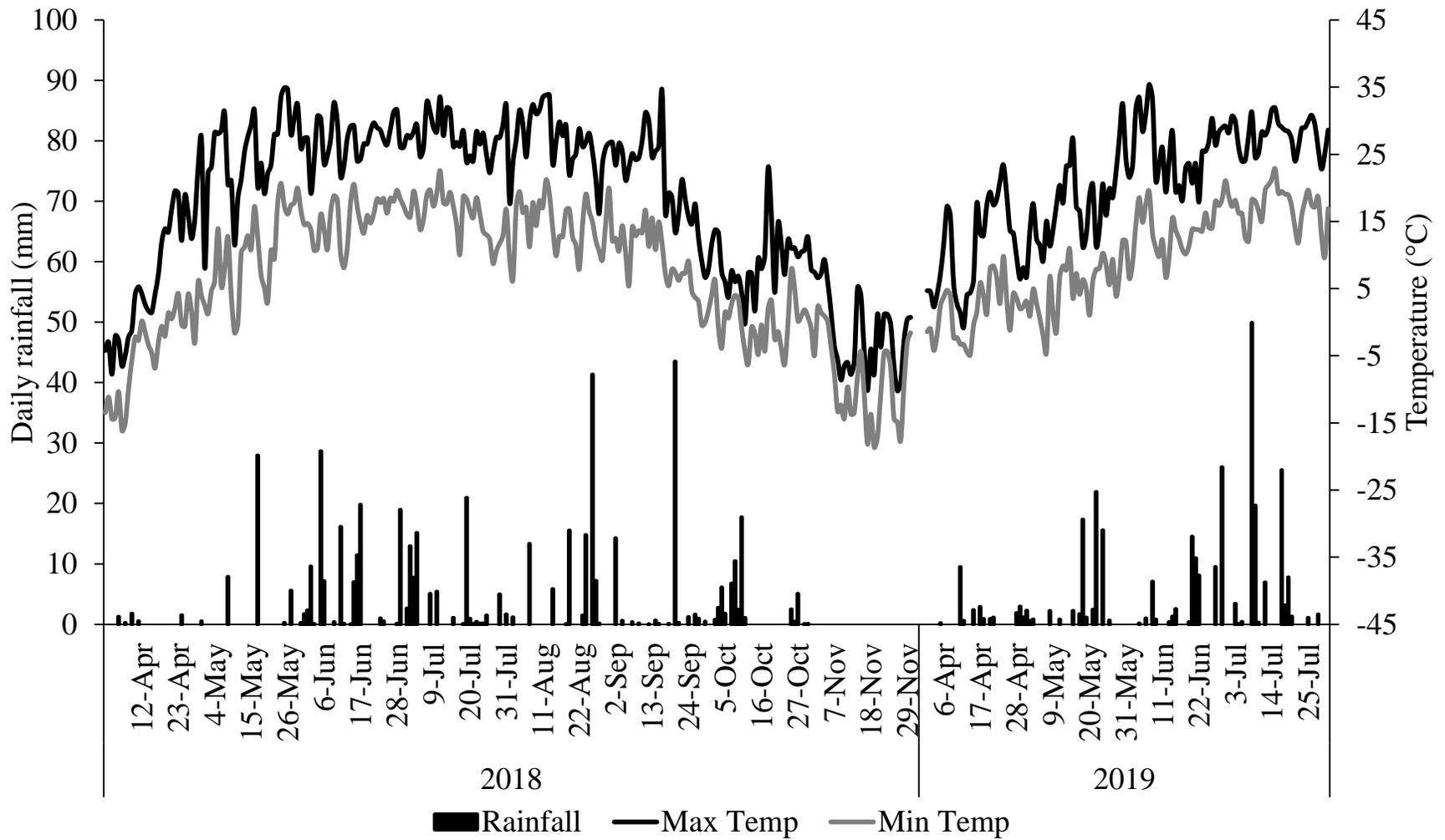
Fig. 2. Daily total rainfall and maximum and minimum temperature for Fargo in 2018 to 2019.

The fall and summer in Prosper in 2018-2019 was marked considerably by more rainfall than the 30-yr average except for the month of July, which was 23 mm below the 30-yr average (Table 7, Fig. 3). In August, rainfall was above the 30-yr average by 12 mm, while in September and October rainfall was 5.4 and 4.9 mm above the normal average respectively (Table 7). Summer and fall average temperatures were below the 30-yr average for the months of July, August, September, and October. The following spring, maximum temperatures for the months of April and May where 3.4 and 3.3°C below the 30-yr average, respectively. Average temperature was also below the 30-yr average for April and May by 1.3 and 2.8°C, respectively. This lower average maximum temperature resulted in decreased GDD accumulation to GDD to 50% flowering (Table 8).  Rainfall for months of April and May were below the 30-yr average as well and even further behind that of Fargo at 14 and 18 mm, respectively for those months. This dry spell did not continue into June in Fargo, but reversed and resulted in a 22 and 68 mm above the 30-yr rainfall for June and July, respectively.

Table 7. Total monthly rainfall, temperature, and departure from normal for the environment at Prosper in 2018 and 2019.

| Environment | Month | Rainfall | | Temperature | | | |
| | | Total | ±Normal[a] | Max. | ±Normal[a] | Min. | ±Normal[a] |
|---|---|---|---|---|---|---|---|
| | | ----------mm--------- | | ---------------------°C---------------------- | | | |
| Prosper 2018 | April | 3.8 | -33.0 | 5.5 | -7.7 | -5.6 | -5.1 |
| | May | 53.9 | -23.6 | 25.0 | 4.4 | 8.7 | 2.5 |
| | June | 79.3 | -21.1 | 26.8 | 1.5 | 14.2 | 2.1 |
| | July | 65.3 | -22.6 | 26.9 | -1.1 | 13.6 | -0.8 |
| | Aug | 78.5 | 12.0 | 26.7 | -0.8 | 12.0 | -1.1 |
| | Sept | 70.9 | 5.4 | 20.9 | -1.1 | 7.4 | -0.2 |
| | Oct | 66.6 | 4.9 | 9.0 | -4.8 | -1.4 | -2.2 |
| | Nov | - | - | -2.0 | -5.4 | -10.1 | -2.9 |
| | | | | | | | |
| Prosper 2019 | April | 23.2 | -13.6 | 9.8 | -3.4 | 0.3 | 0.8 |
| | May | 60.0 | -17.5 | 17.3 | -3.3 | 4.0 | -2.3 |
| | June | 122.0 | 21.7 | 26.0 | 0.7 | 12.3 | 0.2 |
| | July | 156.1 | 68.2 | 28.1 | 0.1 | 15.6 | 1.2 |

Weather data obtained from: https://ndawn.ndsu.nodak.edu/weather-data-monthly.html
[a]Based on 1981-2010 long-term averages

Table 8. Growing degree days (GDD) for Prosper, ND in 2018 and 2019.

| Sowing date | Sowing to end of fall GDD[a] | GDD 1 April to 50% flowering | GDD 1 April to Harvest | Total GDD |
|---|---|---|---|---|
| | ------------------------------------°C d ------------------------------------ | | | |
| Date 1 | 838 | - | - | - |
| Date 2 | 599 | 411 | 1149 | 1748 |
| Date 3 | 362 | 244 | 1149 | 1511 |
| Date 4 | 208 | 258 | 1149 | 1357 |
| Date 5 | 37 | 313 | 1149 | 1186 |
| Date 6 | 11 | - | - | - |

[a] GDD base temperature = 4°C
Last date in in fall was determined by hard freeze below -7°C which occurred on November 7.
Spring and total GDDs were only calculated for experimental units that survived the winter.
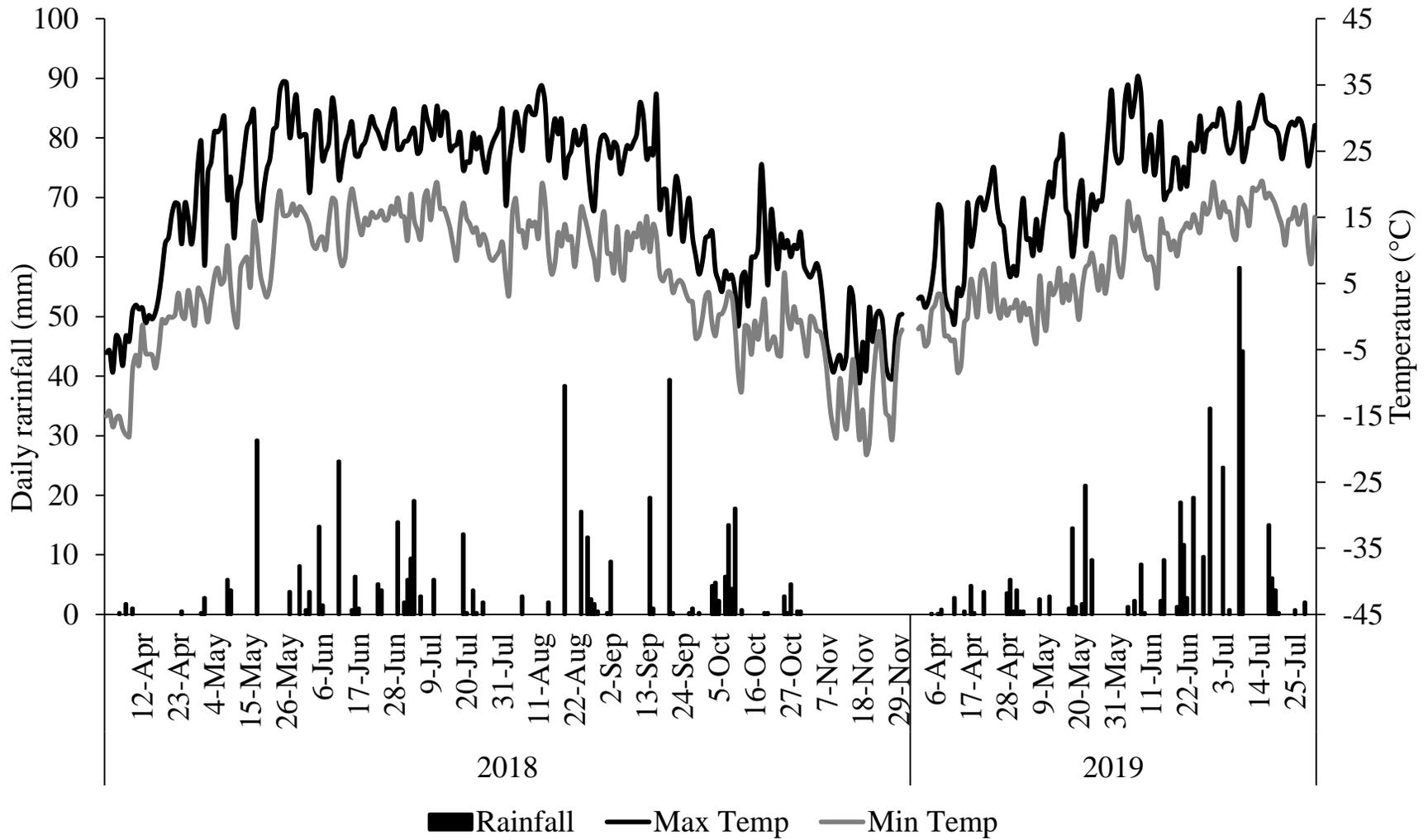
Fig. 3. Daily total rainfall and maximum and minimum temperature for Prosper in 2018 to 2019.

*3.3.2. Fall Stand*

The combined analysis of variance across two environments in the 2018 to 2019 growing season indicated that there was significant effect of sowing date on fall stand establishment ($P \leq 0.05$) (Table 9). Delaying the sowing date resulted in increased stand except for the last sowing date in mid-October.  In Prosper, none of the experimental units of the last sowing (Date 6) had plants established in the fall. (Table 10). The highest fall stand was achieved on Date 5 (Table 10), which likely could be attributed to cooler temperatures than the 30-yr average (Fig. 2, Fig. 3), and 11.4 mm and 12.4 mm rainfall in five days after sowing in Fargo and Prosper respectively (Fig. 2, Fig. 3), as well as less days between sowing and stand counting, as it is likely that in earlier sowing dates some loss of seedlings occurred between sowing and when stands were counted, however, no plant counts were taken at emergence.  Above average rainfall in the 2018 to 2019 growing season, likely increased fall stand establishment of sowing dates 3, 4, 5, and 6. Sowing dates 3 to 6 occurred during periods of increased frequency and amount of rainfall (Fig. 2, Fig. 3). In Connecticut, Zhang and Auer (2019) reported fall stands of 186 to 217 plants m$^{-2}$ in several *Camelina* species. Fall stand counts and subsequent counts are likely influenced both by soil water content at the time of sowing and also rainfall soon after sowing (McVay and Khan, 2011; Gesh et al., 2017; Peterson et al., 2019).

Table 9. Analysis of variance and mean squares for fall stand, gravimetric water content at sowing, pure live seed establishment (PLSE), fall biomass, fall biomass N content, fall biomass N accumulation, and fall soil nitrate at two depths (0-15 cm and 15-60 cm), averaged across two environments (Env) in the fall of 2018. Spring stand and winter survival averaged across two environments in spring 2019.

| SOV | df | Fall stand | PLSE | Soil water | df | Biomass yield | df | Biomass N | Biomass N accum. | df | Fall soil nitrate 0-15 | Fall soil nitrate 15-60 | Spring stand | Winter survival |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Env | 1 | 103933* | 2121* | 1769.77 | 1 | 224304 | 1 | 243* | 544 | 1 | 13454* | 220269* | 1961 | 1752* |
| Rep (Env) | 6 | 5018 | 102 | 0.53 | 6 | 166403 | 6 | 48 | 166 | 6 | 214 | 6104* | 1434 | 1185* |
| Sowing date | 5 | 100677* | 2055* | 24.92* | 4 | 601531 | 4 | 28* | 546 | 5 | 529 | 1650* | 11630* | 5839* |
| Sowing date x Env | 5 | 11282 | 230 | 0.73 | 3 | 382160 | 2 | 20 | 918 | 5 | 288 | 297 | 1423 | 791 |
| Error | 30 | 6395 | 130 | 0.67 | 21 | 142482 | 9 | 30 | 179 | 30 | 284 | 1352 | 788 | 402 |
| CV, % | | 66 | 66 | 2.74 | | 130 | | 19 | 88 | | 74 | 27 | 82 | 71 |

* Significant at 0.05 probability.

Table 10. Mean fall stand, pure live seed establishment (PLSE), soil gravimetric water content, biomass yield, biomass N concentration, and biomass N accumulation averaged across two environments, Fargo and Prosper, ND, in the fall of 2018.

| Sowing date | Fall stand | PLSE | Soil water | Biomass yield | Biomass N | Biomass N accumulation |
|---|---|---|---|---|---|---|
| | Plants m$^{-2}$ | % | g kg$^{-1}$ | kg ha$^{-1}$ | g kg$^{-1}$ | kg ha$^{-1}$ |
| Date 1 | 18 | 3 | 283 | 733 | 27 | 26 |
| Date 2 | 22 | 3 | 279 | 254 | 31 | 10 |
| Date 3 | 124 | 18 | 305 | 221 | 27 | 8 |
| Date 4 | 164 | 23 | 287 | 100 | 29 | 7 |
| Date 5 | 318 | 45 | 316 | - | - | - |
| Date 6 | 75 | 11 | 320 | 0 | - | - |
| LSD (0.05) | 137 | 20 | 11 | NS[a] | NS | NS |

Fall biomass N and N accumulation mean values for dates 1, 2, 3, and 6 include $n=7$, $n=5$, $n=2$, and $n=4$ observations, respectively, since some plots did not have enough biomass for analysis. All other dates have $n=8$.
[a] not significant

### 3.3.3. Pure Live Seed Establishment (PLSE)

The combined analysis of variance across two environments in 2018 indicated that there was a significant effect of sowing date on PLSE (Table 9). A large percentage of the seed sown did not establish, ranging from 3 to 45% of the PLS sown from a total 700 PLS per m$^2$ sown in each experimental unit (Table 10). The first two sowing dates had the same PLSE, at 3%. Sowing dates 3 and 4 had statistically similar stands and PLSE. Sowing date 5 had the highest PLSE and was significantly different from all other sowing dates (Table 10). These results are similar to fall stand and likely a result of cooler temperatures and increased rainfall after sowing (Fig. 2, Fig. 3). Above 30-yr average rainfall in the fall of 2018 likely increased fall stand establishment and thus PLSE in sowing dates 3, 4, 5, and 6. Lower average temperatures in the fall of 2018 probably kept the top soil moist which could have improved germination and plant growth except in October when temperatures were 3.5°C below the 30-yr average, often never reaching above 16°C for optimum germination of camelina (Russo et al., 2010), and often falling below freezing (0°C) overnight and likely prevented germination of the seeds sown on last

sowing date in Prosper. Previous research has demonstrated that sowing rates of winter camelina ($\geq$5.6 kg ha$^{-1}$) tend to be greater than recommended rates for summer camelina (3.4 to 5.6 kg ha$^{-1}$) which is largely assumed due to the harsh climate that seeds must germinate and survive in the fall and over the winter (Gesch and Cermak, 2011; Gesch, 2014; Gesch et al., 2018).

*3.3.4. Soil Water Content at Sowing*

The combined analysis of variance across two environments in 2018 indicated that there was a significant effect of the sowing date on soil gravimetric water content at sowing (Table 9). Soil gravimetric water content was significantly higher at sowing dates 3, 5, and 6 compared with sowing dates 1, 2, and 4 in 2018 (Table 10). This higher soil gravimetric water content can be explained by the increased rain frequency and total rainfall that was recorded prior to sowing of dates 3, 5, and 6 (Fig. 2, Fig. 3). Prior to sowing date 4 was a period of less rainfall when compared with the period prior to sowing dates 3, 5, and 6 (Fig. 2, Fig. 3). Similar to summer camelina in which timely precipitation resulted in increased soil moisture and thus higher stand counts (Gesch, 2014). In irrigation trials, plant stand counts were not significant between different levels of soil water depletion (Hunsaker et al., 2011).

*3.3.5. Fall Biomass Yield*

The combined analysis of variance across two environments in the fall of 2018 indicated that there was not a significant effect of sowing date on fall biomass yield (Table 9). Sowing date average fall biomass varied greatly between sowing dates ranging from 100 to 733 kg ha$^{-1}$ across both environments in the fall of 2018 (Table 10). More biomass was present in earlier sowing dates not because of increased stand but because the plants grew taller with larger leaf area but without fully bolting. In winter canola, sowing earlier has been demonstrated to increase crown height and biomass yield (Holman et al., 2011). This increased crown height is the result of a

longer hypocotyl, which moves the apical meristem further away from the soil surface, exposing it to freezing temperatures during the winter and likely reducing winter survival. On Date 6, in Fargo, plants were in the cotyledon stage (BBCH scale 100) and in Prosper they had not emerged (BBCH scale ≤ 008) yet at the time of the hard frost (-7°C) on 7 November in both Fargo and Prosper.

*3.3.6. Fall Biomass Nitrogen Concentration*

The combined analysis of variance across two environments in the fall of 2018 indicated that there was a not significant effect of sowing date on biomass N concentration (Table 9). Biomass N concentration was not different among the first three sowing dates (Table 10). Plants all remained in the vegetative stage in the fall. The need for N is minimal in vegetative stages compared with reproductive stages, which has been demonstrated with canola (Canola Council, 2017).

*3.3.7. Fall Biomass Nitrogen Accumulation*

The combined analysis of variance across two environments in 2018 indicated that there was not a significant effect of sowing date on fall biomass N accumulation, however indicated the sowing date x environment (Table 9). Fall biomass N accumulation ranged from 7 to 26 kg N ha$^{-1}$ among the first four sowing dates where biomass was collected and analyzed (Table 10). While biomass N accumulation is known to increase as plants develop because of increased biomass growth and N assimilation into the biomass, at the end of the fall all plants were in the vegetative growth stage, as they require a distinct vernalization period for reproductive initiation. Fall biomass nitrogen accumulation values reported in this experiment were considerably higher than those reported by Appelgate et al. (2017), in their study winter camelina was sown after soybean harvest in mid- to late-October in Minnesota, thus there was limited time for camelina to

produce above- and belowground biomass and take up soil nitrogen. Camelina intersown into standing soybean at the R6 stage, produces a fall biomass N accumulation of 29 kg N ha^{-1} (Peterson et al., 2019).

*3.3.8. Fall Soil Nitrate*

The combined analysis of variance across two environments in the fall of 2018 indicated that there was not a significant effect of sowing date on fall soil $NO_3$-N at the 0- to 15-cm depth (Table 9). Lack of differences at the 0- to 15-cm depth can be explained by minimal development of root mass in plants from all sowing dates, since plants in all experimental units were in vegetative rosette growth stages. Previous research has demonstrated camelina to have a higher shoot/root ratio than canola at physiological maturity and negatively affected by rates of N above 75 kg ha^{-1} (Gao et al., 2018). Earlier sowing dates had decreased fall stand establishment with plants being distanced from each other (Table 10) limiting the amount of soil $NO_3$-N at the 0- to 15-cm depth they were able to take up (Table 11). Plants from sowing dates 5 and 6 they were small by hard frost time, thus limiting their ability to take up soil residual $NO_3$-N. Sowing date 3 and 4 had increased fall stands increasing plants ability to effectively take up $NO_3$-N (Table 10). In previous research, camelina root biomass was reported to be concentrated in the top 30-cm of soil at the reproductive stages (BBCH 605 to 609) with 82% of total root mass analyzed (Gesch and Johnson, 2015).

The combined analysis of variance across two environments in the fall of 2018 indicated that there was a significant effect of sowing date on fall soil $NO_3$-N, at the sampling depth of 15- to 60-cm (Table 9). The highest residual soil $NO_3$-N at the 15- to 60-cm depth was at later sowing dates 5 and 6 (Table 11). Later sowing dates were limited in their development to effectively take up soil residual $NO_3$-N from the top 15-cm, which may have resulted in

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increased downward movement of $NO_3$-N to the 15-60 cm soil layer, similar to previous results

reported by Turkeltaub et al. (2016). In addition, the site in Fargo where this experiment was

established in 2018 had been fallowed for two years previous to this experiment and before that

it had alfalfa (*Medicago sativa* L.) for four years, which would explain the high concentration of

soil $NO_3$-N in the 15-60 cm layer. In Prosper, the soil also was fallowed for the season previous

to the experiment and the crop before was soybean.

Table 11. Mean fall soil residual $NO_3$-N levels in the fall of 2018, across two environments,
Fargo and Prosper in North Dakota.

| Sowing date | $NO_3$-N 0-15-cm depth | $NO_3$-N 15-60-cm depth |
|---|---|---|
| | kg ha$^{-1}$ | kg ha$^{-1}$ |
| Date 1 | 25 | 123 |
| Date 2 | 21 | 119 |
| Date 3 | 13 | 127 |
| Date 4 | 14 | 142 |
| Date 5 | 33 | 157 |
| Date 6 | 29 | 141 |
| LSD (0.05) | NS | 22 |

All dates have *n*=8.

### 3.3.9. Spring Camelina Stand

The combined analysis of variance across two environments in 2019 indicated that there

was a significant effect of sowing date on spring camelina stands (Table 9). Plants from the first

and last sowing date did not overwinter, and only a few plants overwintered in sowing date 2,

only in two experimental units (Table 12). Stands of sowing dates 3, 4, and 5 did not

significantly differ from each other (Table 12). Sowing date 3 had 84 plant m$^{-2}$. In the two

environments in 2019, spring rainfall from 15 April to 15 June rainfall was above average

favoring vegetative development and reproductive growth. In many experimental units, weed

control was excellent, likely due to good early spring growth of camelina, suppressing weeds

throughout the growing season. Suppression of weed growth in the spring by overwintering

camelina has been reported before (Gesch and Cermak 2011; Hoerning et al., 2019; Leclère et

al., 2019).

Table 12. Mean spring camelina stands and winter survival averaged across two environments, Fargo and Prosper, ND, in the spring of 2019.

| Sowing date | Spring Stand | Winter survival |
|---|---|---|
| | Plants m$^{-2}$ | % |
| Date 1 | 0 | 0 |
| Date 2 | 7 | 19 |
| Date 3 | 84 | 67 |
| Date 4 | 75 | 57 |
| Date 5 | 41 | 13 |
| Date 6 | 0 | 0 |
| LSD (0.05) | 48 | 33 |

Winter survival date 2 includes *n*=2. All other dates have *n*=8.

### 3.3.10. Winter Survival

The combined analysis of variance across two environments sown in the fall of 2018

indicated that there was a significant effect of sowing date on winter survival (Table 9). Similar

to spring stands, sowing dates 3 and 4 had the highest winter survival, and were different from

sowing date 5, which only had 13% survival (Table 12). Sowing dates 1, 2, and 6 also had lowest

winter survival values, it should be noted that only two experimental units of sowing date 2

survived the winter and sowing date 6 only emerged in Fargo in the fall of 2018 but no plants

survived the winter (Table 12). Apparently, sowing camelina before September likely stresses

the plant reducing its ability to survive the winter. Crown height which is a measurement of the

length of the hypocotyl elongated above the soil surface, has been demonstrated to get longer

with earlier sowing dates in canola in southwest Kansas (Holman et al., 2011). In this

experiment, camelina plants that were visually taller had severely decreased winter survival,

however, since no crown heights were determined we cannot confirm this. While in northeast

Kansas no significant relationship was seen between winter canola crown height and winter

survival (Assefa et al., 2014). Assefa et al. (2014) also suggested that sowing winter canola early

is beneficial to increase growth, however, sowing too early, or sowing to late may be

unfavorable. Significantly shorter hypocotyl length in *Brassica rapa* compared with canola,

possibly allows *B. rapa* to avoid short-term, but not long-term freezing stress (Waalen et al.,

2011). Overwintering stress in the northern Great Plains would be classified as long-term

freezing. Increased plant height and positioning of apical meristems of winter camelina in earlier

sowing dates may explain the lack of winter hardiness. In addition, above average fall

temperatures might reduce winter hardiness. Warmer falls have resulted in excessive growth and

significant winter injury of winter canola in particular with early sowing dates in Indiana

(Christmas, 1996). Higher temperature in the fall of 2017 may have led to excess fall vegetative

growth which may have weakened the plants going into the winter, but since no fall stand counts

or biomass was collected, we cannot confirm this.  Another explanation can be that higher fall

temperatures interfere with the proper cold acclimation needed to tolerate long-term freezing as

demonstrated with winter canola (Trischuk et al., 2014). Winter precipitation, primarily snow

was less in the fall and winter of 2017 and 2018 thus limiting the amount of snow cover,

resulting in less insulation to plants from long exposure to below freezing temperatures as well

allowing for higher soil temperature amplitudes as demonstrated by Rožnoský and Brzezina,

2017. Snow cover has also been demonstrated to result in lower maximum frost depth compared

to no snow cover, which resulted in slower water infiltration and large amount of excess water

on the surface from snow melt (Iwata et al., 2010). Slow water infiltration of snow melt water

and early season rainfall in 2018, likely resulting in increased depth of frost, may have resulted

in increased waterlogging of camelina, which camelina is sensitive to (Gesch and Cermak,

2011). Earlier spring temperatures in the spring of 2018 were also considerably colder than in the

spring of 2019, which may have damaged plants more as they resumed growth. All environments were sown on land that had no previous crop residue, thus limiting the amount of snow catch in each winter, thus exposing plants to winter temperature fluctuations as demonstrated by Rožnoský and Brzezina, 2017. Mean depth of snow covering of 43 cm was demonstrated to prevent fluctuations between 1 cm frozen soil temperature and air temperature, which was likely more snow than what was experienced in the winter of 2017 to 2018 (Sharrat et al., 1992).

*3.3.11. Spring Biomass Yield*

The analysis of the 2018 environment and the combined analysis of variance across two environments in 2019 indicated that there was a significant effect of sowing date on spring biomass yield (Table 13). Spring biomass yield in 2018 (Table 14) was higher than in 2019 (Table 15) which likely could be attributed to the biomass being harvested seven calendar days later as well as having a higher average temperature than the 30-yr average, which resulted in 115 and 91 GDD more for Prosper and Fargo, respectively. Sowing date 5 in the 2018 had greater biomass yield from all other sowing dates in that growing season (Table 14). Sowing dates 3 and 4 spring biomass yield in 2019 were different from each other even though their sowing dates were only 5 (72 GDDs) and 11(154 GDDs) days apart in Fargo and Prosper, respectively (Table 15). This shows the importance of sowing date and how a few days (GDDs) can make a difference later in the spring growth, it is often beneficial to planter earlier in September than into October. (Table 15). In a study where winter camelina was intersown at a different times into standing corn, results produced higher spring biomass yield in later dates (Berti et al., 2017a). All winter camelina plants established before August into corn did not survive the winter, (Berti et al., 2017a) just as was observed in this experiment with sowing dates 1 and 2 sown in Fargo in 2017 and sowing date 1 sown in Fargo and Prosper in 2018.

Table 13. Analysis of variance and mean squares for spring biomass yield, biomass N concentration, biomass N accumulation in 2018 in Fargo and for spring stand, biomass yield, biomass N concentration, biomass N accumulation, spring soil nitrate levels at a depth of 0- to 15-cm averaged across two environments (Env), Fargo and Prosper, ND in 2019 and nitrate levels at a depth 15- to 60-cm depth in Fargo, ND, in 2019.

| SOV | df | Spring 2018 Biomass yield | Biomass N content | Biomass N accumulation | df | Spring 2019 Biomass yield | df | Biomass N content | Biomass N accumulation | df | Soil nitrate 0-15 cm | Soil nitrate 15-60 cm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Env | - | - | - | - | 1 | 8146 | 1 | 513.3* | 3 | 1 | 1063 | - |
| Rep (Env) | 3 | 29967 | 1 | 158 | 6 | 11933 | 6 | 43.9* | 63 | 6 | 721 | 5119* |
| Sowing date | 4 | 479869* | 6 | 863 | 5 | 227870* | 5 | 2.7 | 33 | 3 | 147* | 1469* |
| Sowing date x env | - | - | - | - | 3 | 6789 | 3 | - | - | 3 | 16 | - |
| Error | 12 | 4419 | 18 | 232 | 18 | 12396 | 16 | 0.3 | 13 | 10 | 335 | 236 |
| CV, % | | 65 | 8 | 51 | | 93 | | 1.4 | 20 | | 45 | 6 |

* Significant at 0.05 probability

Table 14. Mean spring biomass yield, biomass N concentration, and biomass N accumulation in Fargo, ND, in 2018.

| Sowing date | Biomass yield | Biomass N concentration | Biomass N accumulation |
|---|---|---|---|
| | kg ha$^{-1}$ | g kg$^{-1}$ | kg N ha$^{-1}$ |
| Date 1 | 0 | - | - |
| Date 2 | 0 | - | - |
| Date 3[a] | - | - | - |
| Date 4 | 343 | 55 | 19 |
| Date 5 | 827 | 56 | 46 |
| Date 6 | 451 | 53 | 24 |
| LSD (0.05) | 325 | NS[b] | NS |

[a] Sowing date 3 was skipped due to excessive soil moisture. All dates have $n=4$.
[b] not significant

Table 15. Mean spring biomass, N concentration, and biomass N accumulation averaged across two environments, Fargo and Prosper, ND, in 2019.

| Sowing date | Biomass yield | Biomass N concentration | Biomass N accumulation |
|---|---|---|---|
| | kg ha$^{-1}$ | g kg$^{-1}$ | kg N ha$^{-1}$ |
| Date 1 | 0 | - | - |
| Date 2 | - | - | - |
| Date 3 | 370 | 36 | 16 |
| Date 4 | 565 | 46 | 26 |
| Date 5 | - | - | - |
| Date 6 | 0 | - | - |
| LSD (0.05) | 188 | NS[a] | NS |

Spring biomass for dates 2, 3, and 4 include $n=2$, $n=6$, and $n=7$ respectively; All other dates have $n=8$. N concentration and N accumulation for dates 3, and 4 include $n=6$, and $n=2$, respectively since some plots did not have enough biomass for analysis.
[a] not significant

*3.3.12. Spring Biomass Nitrogen Concentration*

The analysis of variance of the 2018 environment and the combined analysis across two

environments in 2019 indicated that there was not a significant effect of sowing date on spring

biomass nitrogen concentration (Table 13). Nitrogen concentration ranged from 53 to 56 g kg$^{-1}$

in 2018, which is higher than what has been reported by Appelgate et al., (2017) (Table 14).

Only two sowing dates had biomass for analysis in 2019, with N concentration ranging from 36

to 46 g kg$^{-1}$ similar to the 2018 biomass are higher than what has been reported by Appelgate et al., (2017) (Table 15).  In 2018, spring biomass N concentration was considerably higher than in 2019 (Table 15), which too could be attributed to delayed harvest of the biomass, as plants had more time to take up more N from the soil. Research conducted in Morris, MN, has demonstrated very similar results in N concentration not only in spring biomass N concentration in winter camelina but also in field pennycress biomass (Weyers et al., 2019).

*3.3.13. Spring Biomass Nitrogen Accumulation*

The analysis of the 2018 environment and combined analysis of variance across two environments in 2019 indicated that there was not a significant effect of sowing date on spring biomass nitrogen accumulation (Table 13). Highest total biomass N accumulation was 46 kg N ha$^{-1}$ in sowing date 5, while sowing dates 4 and 6 had 19 and 24 kg N ha$^{-1}$, respectively in 2018 (Table 14). In 2019, sowing dates 1 and 6 plants did not overwinter (Table 12). Sowing date 4 did have significantly higher biomass harvested, but not significantly higher N concentration than sowing date 3, resulting in not significantly different N accumulation (Table 15).  Previous results have shown that spring biomass N accumulation was often less than what was reported in this experiment but this could be attributed to the difference in the amount of biomass harvested rather than N content (Appelgate et al., 2017). Weyers et al. (2019) reported that camelina and field pennycress accumulated a minimum of 50 kg ha$^{-1}$ at the start of intercropping with soybean after nitrogen fertilizers were applied.

*3.3.14. Spring Soil Nitrate*

The combined analysis of variance across two environments in spring 2019 indicated that there was significant effect of sowing date on spring residual soil NO$_3$-N levels at the 0- to 15-cm and analysis of the Fargo environment indicated that 15- to 60-cm depth was significant

(Table 13). Soil residual $NO_3$-N samples in Prosper at 15- to 60-cm depth could not be taken due to excessive soil moisture, which made sample extraction impossible. At both depths, soil residual $NO_3$-N tended to be higher in later sowing dates. The largest amount of residual soil $NO_3$-N at the 0- to 15-cm depth was present at sowing date 5 with 67 $NO_3$-N kg ha$^{-1}$ (Table 16) which was considerably higher than what was reported the previous fall at 33 $NO_3$-N kg ha$^{-1}$ (Table 11). Changes from the fall to spring sampling of soil $NO_3$-N could be attributed to increased mineralization of soil organic matter, release of N from decaying winter camelina plants and weeds, and downward movement of $NO_3$-N into the soil profile. All sowings in 2018 that survived the winter were significantly different in soil $NO_3$-N from each other except, sowing dates 2 and 3 at the 0- to 15-cm depth (Table 16). While at the 15- to 60-cm depth sowing dates 4 and 5 were the same (Table 16). At the 15- to 60-cm depth soil residual $NO_3$-N ranged from 195 to 266 kg ha$^{-1}$, with less being present at earlier sowing dates. The large amount of soil residual $NO_3$-N is a result of the Fargo location being fallowed for two previous years and prior to that with alfalfa. While in Prosper, the soil was fallowed the previous season and prior to that soybean was produced. Appelgate et al. (2019) reported that camelina was not significantly different from the control soil residual $NO_3$-N concentration in the spring. Oppositely, in this experiment given that are differences between sowing dates, it is likely that camelina did reduce soil residual $NO_3$-N if compared with a fallowed area.

Table 16. Mean soil residual NO$_3$-N levels in the spring of 2019, across two environments Fargo and Prosper at the 0- to 15-cm-depth and one environment at the 15- to 60-cm-depth.

| Sowing date | N0$_3$-N 0-15-cm depth | NO$_3$-N 15-60-cm depth |
|---|---|---|
| | kg ha$^{-1}$ | kg ha$^{-1}$ |
| Date 1 | - | - |
| Date 2 | 30 | 195 |
| Date 3 | 24 | 223 |
| Date 4 | 39 | 261 |
| Date 5 | 66 | 266 |
| Date 6 | - | - |
| LSD (0.05) | 9 | 35[a] |

Soil NO$_3$-N at 0-15-cm depth for dates 2 and 5 include $n=2$, and $n=6$ respectively, all other values $n=8$. Soil NO$_3$-N at the 15-60-cm depth for dates 2 include $n=1$, all other dates $n=4$.
[a] LSD for analysis of Fargo 2019

*3.3.15. Flowering Date*

The analysis of the 2018 environment indicated that there was a significant effect of sowing date on days required to reach 50% flowering (Table 17). However, the combined analysis of variance across two environments in 2019 was not significant for sowing date effect (Table 17). Days required to reach 50% flowering in 2018 (Table 18) were less than at both environments in 2019 (Table 18). The earliest sowing date to reach 50% flowering in 2018 was 10 days sooner than the earliest date in 2019, however GDD difference was only 4 GDD. Differences in days to 50% flowering between the two growing seasons could be explained by the warmer temperatures in the spring of 2018 (Fig. 1). This indicates that timing to 50% flowering is temperature dependent. Of the sowing dates that survived the 2017-2018 winter the days required to reach 50% flowering only varied by 10 days (169 GDDs), with sowing date 4 only requiring 47 days (274 GDDs) and sowing date 6 requiring 57 days (443 GDDs) from 1 April (Table 18). While in 2019, in the experimental units that survived the winter, days required to reach 50% flowering ranged from 57 to 65 days (271 to 368 GDDs) from 1 April a slightly narrower GDD range than in 2018 (274 to 443 GDDs) (Table 18). In both growing seasons, we

can see a trend that as sowing date was delayed so did the days required to reach 50% flowering, but this did not hold true in 2018 sown experimental units (Table 18). Sowing date 2 in 2018 was the latest flowering date recorded, but only reflects two experimental units so should be interpreted cautiously. The longer time to 50% flowering for sowing date 2 in 2019 can be a result of decreased plant stands (Table 12), which allowed the remaining plants to become quite large and branched, which resulted in delayed flowering as plants remained vegetative longer. Gesch and Cermak (2011) reported similar days to reach 50% flowering as what is reported in this experiment.

Table 17. Analysis of variance and mean squares for days to 50% flowering, GDDs to 50% flowering, and harvest stand in 2018, in Fargo and days to 50% flowering, GDDs to 50% flowering, harvest stand, and spring to harvest stand reduction, averaged across two environments (Env) in 2019.

| | | 2018 | | | | | 2019 | | | | | |
| | | Days to 50% | GDD to 50% | | Harvest | | Days to 50% | GDD to 50% | | Harvest | | Spring to harvest stand |
| SOV | df | flowering | flowering | df | stand | df | flowering | flowering | df | stand | df | reduction |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Env | - | - | - | - | - | 1 | 19 | 26096 | 1 | 99 | 1 | 1998 |
| Rep | 3 | 2 | 596 | 3 | 364 | 6 | 9 | 1776 | 6 | 107 | 6 | 15301 |
| Sowing date | 2 | 104* | 40309* | 5 | 7572* | 3 | 33 | 6392* | 5 | 1747* | 3 | 8857 |
| Sowing date x Env | - | - | - | - | - | 3 | 5 | 662 | 5 | 54 | 3 | 18021 |
| Error | 6 | 7 | 1882 | 15 | 527 | 10 | 4 | 757 | 30 | 71 | 10 | 10894 |
| CV, % | | 5 | 13 | | 60 | | 3 | 9 | | 58 | | 441 |

* Significant at 0.05 probability.

Table 18. Mean calendar days after 1 April required to reach 50% flowering and GDDs accumulated from 1 April to 50% flowering in Fargo, ND, in 2018 and across two environments, Fargo and Prosper, ND, in 2019.

| | 2018 | | 2019 | |
|---|---|---|---|---|
| Sowing date | Days to 50% flowering | GDDs to 50% flowering | Days to 50% flowering | GDDs to 50% flowering |
| Date 1 | - | - | - | - |
| Date 2 | - | - | 65 | 310 |
| Date 3[a] | - | - | 57 | 271 |
| Date 4 | 47 | 267 | 59 | 297 |
| Date 5 | 50 | 308 | 63 | 368 |
| Date 6 | 57 | 458 | - | - |
| LSD (0.05) | 5 | 75 | NS[b] | 55 |

All values $n=4$ in 2018. 2019, days to reach flowering for dates 2 and 5 include $n=2$, and $n=6$ respectively. All other dates $n=8$.
[a] In 2018, sowing date 3 was skipped due to excessive moisture
[b] not significant

### 3.3.16. Camelina Plant Stand at Harvest

The analysis of the 2018 environment and combined analysis of variance across two environments in 2019 indicated that there was a significant effect of sowing date on harvest stand (Table 17). In 2018, harvest stands ranged from 0 to 94 plants m$^{-2}$, while in 2019, harvest stands ranged from 0 to 34 plants m$^{-2}$ (Table 19). In 2018, higher stands were obtained with sowing dates 5 and 6 compared with sowing date 3, which had 24 plants m$^{-2}$, while sowing dates 5 and 6 had 75 and 94 plants m$^{-2}$, respectively (Table 19). In 2019, sowing date 3 had higher stands than sowing dates 4 and 5, which had 25 and 23 plants m$^{-2}$, respectively (Table 19). Differences between the two growing seasons could likely be explained by the differences in spring stands counts, because in 2019 spring stand counts (Table 12) exhibited a very similar trend to harvest stand (Table 19). Higher harvest stands in 2018 may have been a result of drier spring conditions (Fig. 1) since winter camelina is susceptible to waterlogging stress (Gesch and Cermak, 2011). Gesch and Cermak (2011) also reported increased stand in no-tilled conditions

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compared with a chisel-plowed field, which may explain decreased stand in the fall, spring, and at harvest time in this experiment which was seeded into fallow no-tilled conditions.

Table 19. Mean harvest stand in Fargo, ND, in 2018 and harvest stand and spring to harvest stand reduction averaged across two environments, Fargo and Prosper, ND in 2019.

| Sowing date | Harvest stand 2018 | Harvest stand 2019 | Spring to harvest stand reduction |
|---|---|---|---|
| | Plants m$^{-2}$ | Plants m$^{-2}$ | % |
| Date 1 | 0 | 0 | - |
| Date 2 | 0 | 5 | 6 |
| Date 3[a] | - | 34 | 0 |
| Date 4 | 24 | 25 | 57 |
| Date 5 | 75 | 23 | 26 |
| Date 6 | 94 | 0 | - |
| LSD (0.05) | 35 | 9 | NS[b] |

Harvest stand in 2018, all dates $n=4$. Spring to harvest stand reduction for dates 2, and 5 include Number of observations $n=2$ and $n=6$ respectively. All other dates $n=8$.
[a] In 2018, sowing date 3 was skipped due to excessive moisture
[b] not significant

### 3.3.17. Spring to Harvest Stand Reduction

The combined analysis of variance across two environments in 2019 indicated that there was not a significant effect of sowing date on spring to harvest stand reduction (Table 17). Stand reduction was 57% in sowing date 4 and no stand reduction was observed in sowing date 3 (Table 19). The large reduction from spring to harvest experienced with sowing date 4 is likely partially attributable to using different locations within the experimental units at different measurement times as a result of not marking locations and uneven stand similar to what was reported by Waraich et al., (2013).

### 3.3.18. Plant Height at Harvest

The analysis of the 2018 environment and combined analysis of variance across two environments in 2019 was not significant for sowing date on plant height at harvest (Table 20). In 2018, plants were shorter than in 2019 (Table 21). In 2018, plant height ranged from 54 to 74

cm, while in 2019 heights ranged from 74 to 83 cm. A likely contributing factor to the differences in plant height between the two growing seasons was the presence of large amount of soil residual $NO_3$-N in the soil (Table 1) in Fargo and Prosper in 2018 as well as more favorable growing conditions, though large amounts of soil residual $NO_3$-N were also present in Fargo in 2017. Gesch et al. (2018) reported that plant height at harvest was influenced by cultivar and to a lesser degree, by sowing rate and date, which can explain the lack of differences in this experiment as only one cultivar was used. Plant height is a very important plant trait when evaluating winter camelina cultivars for relay-crop systems (Gesch et al., 2014; Berti et al., 2015), where a greater plant height difference between the two crops at the time that camelina is harvested is beneficial to prevent damage to the undersown crop.

Table 20. Analysis of variance and mean squares for plant height, 1000-seed weight, seed yield, and harvest index in 2018, in Fargo and averaged across two environments (Env), Fargo and Prosper, ND, in 2019.

| SOV | | 2018 | | | | | | 2019 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | Plant height | 1000-seed weight | df | Seed yield | Harvest index | df | Plant height | 1000-seed weight | df | Seed yield | Harvest index |
| Env | | | | | | | 1 | 96 | 0.049* | 1 | 43220 | 508* |
| Rep (Env) | 3 | 8 | 0.0036 | 3 | 14378 | 17 | 6 | 21 | 0.009* | 6 | 354384 | 11 |
| Sowing date | 2 | 68 | 0.0029 | 4 | 205902* | 64* | 3 | 50 | 0.019 | 5 | 345638 | 25 |
| Sowing date x env | | | | | | 8 | 3 | 87 | 0.021* | 5 | 96829 | 25 |
| Error | 6 | 18 | 0.004 | 12 | 24416 | | 10 | 75 | 0.001 | 30 | 256869 | 9 |
| CV, % | | 8 | 5.3166 | | 44 | 16 | | 11 | 3.197 | | 46 | 13 |

* Significant at 0.05 probability.

Table 21. Mean plant height, 1000-seed weight, yield, and harvest index of sowing dates in Fargo, in 2018, and averaged across two environments, Fargo and Prosper, ND, in 2019.

| | 2018 | | | | 2019 | | | |
|---|---|---|---|---|---|---|---|---|
| Sowing date | Plant height | 1000-seed weight | Seed yield | Harvest index | Plant height | 1000-seed weight | Seed yield | Harvest index |
| | cm | g | kg ha$^{-1}$ | % | cm | g | kg ha$^{-1}$ | % |
| Date 1 | - | - | - | - | - | - | - | - |
| Date 2 | - | - | - | - | 83 | 1.01 | 680 | 21 |
| Date 3[a] | - | - | - | - | 77 | 1.06 | 1317 | 21 |
| Date 4 | 47 | 1.22 | 99 | 12 | 77 | 1.03 | 1176 | 25 |
| Date 5 | 54 | 1.26 | 421 | 19 | 74 | 0.96 | 840 | 20 |
| Date 6 | 54 | 1.28 | 536 | 19 | - | - | - | - |
| LSD (0.05) | NS[b] | NS | 171 | 4 | NS | NS | NS | NS |

[a] Sowing date 3was skipped due to excessive moisture

In 2018 all values have $n=4$. In 2019, plant height, 1000-seed weight, seed yield, and Harvest index for dates 2, and 5 include $n=2$, and $n=6$ respectively, All other dates have $n=8$.

[b] not significant

*3.3.19. Thousand-Seed Weight*

The analysis of the 2018 environment and combined analysis of variance across two environments in 2019 indicated that there was not a significant effect of sowing date on 1000-seed weight (Table 20). Thousand-seed weight was higher in 2018 than in 2019 (Table 21). Possibly the high residual soil $NO_3$-N in Fargo explains in part differences in 1000-seed weight, as excess nitrogen can increase1000-seed weight (Jiang and Caldwell, 2016). In addition, thousand-seed weight differences between the two growing seasons could be a result of GDD accumulation differences, especially between Fargo in 2018 and Prosper in 2019 (Table 4, 6, 8). In 2018, plants in all experimental units were dry at the time of harvest, at physiological maturity. Full maturity is defined by Martinelli and Galasso (2011) when nearly all the silicles contain seeds that are of a deep yellow/orange color and hard. Physiological maturity occurs when maximums dry weight of camelina seed is reached prior to full plant ripening (Walia et al., 2018). However, in 2019, in Prosper, grasshoppers (*Acrididae:Melanoplinae*) moved into experimental units prior to plant dry drown and consumed a majority of the green leaves, thus limiting the flow of nutrients to the developing seed, as a result plant dry down was faster than anticipated and allowed for earlier harvest. Adult grasshopper damage to canola by adults is especially a concern at pod development (Kandel et al., 2019). In Fargo, in 2019, dry down of plants was slowed down by frequent rainfall especially in July. The seed shattering observed was due to suspected avian predation, and led us to harvest before adequate plant dry down and before full physiological maturity of all seeds, which may have resulted in the observed decrease in 1000-seed weight. Camelina usually does not shatter as easily as canola and field pennycress, but bird damage can cause heavy shattering (Walia et al., 2018). Previous research has also demonstrated that sowing date, cultivar, and interaction between cultivar and sowing date can be

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significant for 1000-seed weight of summer camelina in Chile (Berti et al., 2011). Camelina 1000-seed weight has been reported to range between 0.8 and 1.8 g (Berti et al., 2016).

*3.3.20. Seed Yield*

The analysis of the 2018 environment indicated that there was a significant effect of sowing date on seed yield (Table 20). However, the combined analysis of variance across two environments in 2019 was not significant for seed yield (Table 20). In 2018, seed yield ranged between 99 and 536 kg ha$^{-1}$, while in 2019 seed yield ranged from 170 to 1317 kg ha$^{-1}$ (Table 21). Harvest stand counts and seed yield in 2018 followed a similar trend (Table 19) pointing to similar yield per plant even with increasing stand density. The season-long moisture deficiency in the Fargo in 2017 hindered adequate stand establishment and vegetative growth in the fall. The spring of 2018 also was below the 30-yr average rainfall for the months of April and May by 29 and 28mm, respectively. While in June and July, rainfall was above the 30-yr average by 24 and 10mm, respectively. The decrease in early-season rainfall may have resulted in increased stress for the plants when they were entering reproduction phase. The increased rainfall in June was beneficial as the plants were in the reproductive period. The above average rainfall in July was not as needed for plants as they were already starting to mature. The highest yielding sowing date in 2018 (Date 6) was less than half of the highest seed yield in 2019. Differences between the two years could be attributed to the lack of early season rainfall in the spring, which likely stressed plants and hastened plant maturity. In 2019, seed yield was 1317 and 1176 kg ha$^{-1}$ in sowing dates 3 and 4, respectively (Table 21). Sowing dates 3 and 4 were only five days apart in Fargo and 11 days apart in Prosper, corresponding to 72 and 154 GDD, respectively, which might explain their similar response (Table 2). Sowing date 2 in 2019 had a mean seed yield of 680 kg ha$^{-1}$ (Table 21). Similar to the effect on 1000-seed yield, grasshoppers moved into

experimental units in Prosper in 2019, approximately on 10 July, and consumed a majority of the

green leaves limiting the flow of nutrients into the developing seeds and reducing both 1000-seed

weight and seed yield. In 2019, in Fargo, shattering reduced seed yield not only by seed lost prior

to harvest but also by forcing us to harvest before physiological maturity to limit any further seed

losses. Seed yield in all three environments were likely influenced by both fall and spring stands

as they were significant in both environments established in the fall of 2018. The number of

shattered silicles were not affected by sowing dates and ranged from 754 to 1324 silicles m$^{-2}$ in

Fargo in 2019 (Table 22, 23).  McVay and Khan (2011) previously demonstrated stand reduction

occurring at bolting did significantly reduced yield, similar to spring to harvest stand reduction in

this experiment, possibly contributing to decreased yields in this experiment.

Table 22. Analysis of variance and mean squares for the number of shattered silicles on soil
surface in Fargo, ND, in 2019.

| SOV | df | Number of shattered silicles on soil |
|---|---|---|
| Rep | 3 | 2233 |
| Sowing date | 3 | 75850 |
| Error | 6 | 111518 |
| CV, % | | 27 |

* Significant at 0.05 probability.


Table 23.  Mean number of shattered silicles on soil surface after harvest in Fargo, ND, in 2019.

| Sowing date | Shattered silicles on soil |
|---|---|
| | no. silicles m$^{-2}$ |
| Date 1 | - |
| Date 2 | 754 |
| Date 3 | 1285 |
| Date 4 | 1324 |
| Date 5 | 1285 |
| Date 6 | - |
| LSD (0.05) | NS[b] |

Shattered silicles on soil dates 2 include *n*=1. All other dates *n*=4
[b] not significant

The substantial amount of rainfall above the 30-yr average in both environments in 2019 probably did not reduce seed yield as plants had completed flowering and seed filling when this excess rainfall occurred, but it delayed harvest by preventing adequate dry down of plants. Harvest dry down was also hindered by the maximum average temperature in July being 28°C (Fig. 2). However, the average temperature in July was 22°C which was 0.9°C above the 30-yr average, but coupled with lower maximum temperature and larger excess of moisture likely prevented dry drown of plants and delayed harvest in Fargo and Prosper in 2019.

*3.3.21. Harvest Index*

The analysis of the 2018 environment indicated that there was a significant effect of sowing date on harvest index (Table 20). However, the combined analysis of variance across two environments in 2019 was not significant for harvest index (Table 20). Harvest index ranged 12 to 19% in 2018 and from 20 to 25 % in 2019. (Table 21). Sowing date 5 and 6 had significantly higher harvest indexes in 2018 both at 19% compared with sowing date 4, which had a harvest index of 12%. Sowing date 4 in 2019 had a harvest index of 25% while sowing date 5 had a harvest index of 20%. (Table 21). Previous research using a summer camelina cultivar in Morris, MN, reported harvest indexes ranging from 25 to 32%, which was considerably less variable among sowing dates than on this study (Gesch et al., 2017). Lower harvest index in the winter camelina biotype has been reported before and can be explained by lesser efforts in winter camelina breeding compared with summer camelina (Berti et al., 2016). Walia et al. (2018) reported that winter camelina harvest index was influenced by harvest date, with later dates having significantly higher harvest index. Delayed harvest in 2019 could have been a contributing factor to higher harvest index values.

*3.3.22. Seed Yield Components*

The combined analysis of variance across two environments in 2019 indicated that there was not a significant effect of sowing date on the seed yield components of total silicles, silicles present and missing, shattered silicles percentage, and seeds per silicle (Table 24). Shattered silicles ranged from 39 to 42% of the total silicles per plant, lower percentages were observed with sowing date 3 and 4 (Table 25). A higher degree of silicles shattering occurred in Fargo at all sowing dates, likely contributed to overall higher combined shattering percentages.

Total silicles, which include ones that were shattered prior to harvest were not significantly affected by sowing date and did not vary to any large degree between sowing dates 3, 4, and 5; however, sowing date 2 had increased total number of silicles, also likely attributable to reduced harvest stand allowing individual plants to become quite large (Table 25). Silicles present or missing did not vary considerably between sowing dates 3, 4, and 5 (Table 26). Seeds per silicle were very similar across all four sowing dates that were harvested, only ranging from 11 to 12 seeds per silicle (Table 26), which is very similar to what is reported in Solis et al. (2011) and Berti et al. (2011). Harvest stand results presented above represent final stand which is also a critical yield component of plants.

*3.3.23. Total Biomass, Seed Yield. and Harvest Index per Plant*

The combined analysis of variance across two environments in 2019 indicated that there was not a significant effect of sowing date on the seed yield components of total biomass, seed yield, and harvest index per plant (Table 24). Total biomass per plant ranged from 14.3 to 35.4 g, with sowing date 2 having the largest amount of biomass present, which likely could be attributed to a significantly lower harvest stand allowing individual plants to get larger in size (Table 25). Seed yield per individual plants did not significantly differ between sowing dates,

ranging from 5.1 to 9.3 g. Sowing date 2 had the highest seed yield, which likely could be

explained by decreased stands that allowed plants to become quite large by with branching and

increased number of silicles (Table 26). Seed yield per plant did not vary considerably except for

sowing date 2, which was 9.3 g a little less than double the yield per plant which is reported for

sowing dates 3, 4, and 5. Harvest index calculated on a per plant basis ranged from 17 to 24%,

compared to harvest index calculated by 2-m from each experimental unit the results are similar.

Table 24. Analysis of variance and mean squares for the number of shattered silicles, total biomass, seed yield, harvest index, total number of silicles, silicles present per plant, silicles missing per plant, and number of seeds per silicle in two environments (Env) in 2019.

| Sources of variation | df | Shattered silicles[a] | Total N° silicles plant$^{-1}$ | N° silicles present plant$^{-1}$ | N° silicles missing plant$^{-1}$ | N° seed silicle$^{-1}$ | Total biomass yield | Seed yield plant$^{-1}$ | Harvest index |
|---|---|---|---|---|---|---|---|---|---|
| Env | 1 | 1.50 | 1441655 | 3074153 | 305407 | 10.4 | 633 | 338.6 | 0.102 |
| Rep (Env) | 6 | 0.04 | 514556 | 234996 | 111073 | 3.3 | 152 | 15.7 | 0.004 |
| Sowing date | 3 | 0.02 | 208411 | 74600 | 35113 | 1.2 | 243 | 8.5 | 0.006 |
| Sowing date x env | 3 | 0.01 | 334415 | 260064 | 5729 | 6.0 | 111 | 74.4 | 0.025 |
| Error | 34 | 0.02 | 143804 | 118726 | 11342 | 2.4 | 71 | 9.9 | 0.004 |
| CV, % | | 39.61 | 51 | 66 | 47 | 13.1 | 50 | 57.9 | 27.687 |

* Significant at 0.05 probability.
[a] Shattered silicles percentage of the total present on the plant

Table 25. Shattered silicles, total biomass, seed yield, and harvest index per plant for six sowing dates averaged across in Fargo and Prosper in 2019.

| Sowing date | Shattered silicles | Total biomass yield | Seed yield | Harvest index |
|---|---|---|---|---|
| | % | g plant$^{-1}$ | g plant$^{-1}$ | % |
| Date 1 | - | - | - | - |
| Date 2 | 42 | 35.4 | 9.3 | 17 |
| Date 3 | 32 | 15.6 | 5.0 | 23 |
| Date 4 | 30 | 14.3 | 5.2 | 24 |
| Date 5 | 39 | 16.1 | 5.1 | 24 |
| Date 6 | - | - | - | - |
| LSD (0.05) | NS | NS | NS | NS |

Shattered, total biomass, grain yield, and harvest index for dates 2, and 5 include *n=4*, and *n=12* respectively, All other values have *n=16*.
[b] not significant

Table 26. Total silicles, silicles present, silicles missing per plant and number of seeds per silicle for sowing dates averaged across two environments, Fargo and Prosper, ND in 2019.

| Sowing date | Total N° silicles plant$^{-1}$ | N° silicles present plant$^{-1}$ | N° silicles missing plant$^{-1}$ | N° seeds silicle$^{-1}$ |
|---|---|---|---|---|
| Date 1 | - | - | - | - |
| Date 2 | 1391 | 925 | 466 | 12 |
| Date 3 | 680 | 490 | 190 | 12 |
| Date 4 | 658 | 482 | 176 | 11 |
| Date 5 | 740 | 490 | 250 | 12 |
| Date 6 | - | - | - | - |
| LSD (0.05) | NS | NS | NS | NS |

Total N° silicles plant$^{-1}$, N° silicles present plant$^{-1}$, N° silicles missing per plant$^{-1}$, and N° seed silicle$^{-1}$ for dates 2, and 5 include *n*=4, and *n*=12 respectively. All other dates have *n*=16.

*3.3.24. Seed Quality*

The analysis of the 2018 environment indicated that there was a significant effect of sowing date on several seed quality characteristics including fatty acids (16:0, 18:1 22:0, and 20:4) and the combined analysis of variance across two environments in the 2019 growing season indicated that there was no significant effect of sowing date on any seed quality characteristics (Table 27, Table, 28). Seed oil concentration was not affected by sowing date in any environment and ranged from 247 to 267 g kg$^{-1}$ in 2018 (Table 26), and from 268 to 294 g kg $^{-1}$ in 2019 (Table 27). In both growing seasons, total oil concentration (Table 29, Table 30) was similar to what has been previously been reported for winter camelina oil concentration (Gesch and Cermak 2011; Gesch and Archer 2013; Gesch et al., 2018; Walia et al., 2018). Seed crude protein concentration did not vary between sowing dates at any environment. Crude protein ranged from 299 to 303 g kg$^{-1}$ in 2018 and from 268 to 275 g kg$^{-1}$ in 2019 environments. Nitrogen concentration was the same for all sowing dates. Higher seed crude protein reported in the 2018 could be attributed to higher temperatures and increased stress in the late spring and early summer on plants, as well as the high number of residual nitrates in the soil (Elferjani and Soolanayakanahally 2018). Elferjani and Soolanayakanahally (2018) also reported a decrease in oil concentration when canola plants were exposed to heat stress. Crude protein levels are within the range of what has been previously reported for several winter camelina cultivars by Gesch et al. (2018). In the 2018 environment, only two fatty acids were different among sowing dates ($P \leq$ 0.05); palmitic and oleic acids (Table 31). Palmitic acid ranged from 6.3 to 6.6% of total oil, while oleic acid ranged form12.1 to 14.2% of total oil in the 2018 environment. In the combined 2019 environments, fatty acids were not different among sowing dates ($P \leq 0.05$) (Table32). Linolenic acid in the 2018 environment ranged from 36.7 to 37.1% of total oil, while in the combined environments in 2019 ranged from 34.3 to 36.3% of total oil.  Overall range of erucic

acid in both the 2018 environment and the combined environments in 2019 was 3.8 to 3.9% of total oil in both years, which is higher than the desired threshold of 2% for food-grade rapeseed oil in the United States (Code of Federal Regulation). Results reported here for fatty acid profile in both growing seasons are similar to what has been reported before for the cultivar Joelle, however values for oleic, and linolenic acids were slightly higher than what was reported in Gesch and Cermak (2011) and Gesch et al. (2018), while linoleic tended to be lower.

3.4. Conclusion

While demonstrating a wide range of acceptable sowing dates, sowing winter camelina in the beginning to middle of September and even as late as early October can have similar seed yield. Fall biomass was not significantly different between sowing dates but ranged from 0 to 733 kg ha$^{-1}$, with more biomass being present at earlier sowing dates. Winter survival ranged from no plants surviving the winter to 67% of plants surviving. Environmental variables such as suitability of winter and cumulative precipitation can have a significant effect on plant stand establishment and persistence to the next spring. Camelina can effectively remove residual soil nitrate at 15-60-cm prior to winter and again the following spring when sown prior to mid-September. Days to 50% flowering tended to increase with latter sowing dates. Depending on the objective, winter camelina used as a cover crop the sowing date is a critical factor, with sowing prior to mid-September critical for biomass production and reducing soil residual nitrate, while sowing in September and even as late as early October for the highest harvestable seed yield in the following growing season.

Table 27. Analysis of variance and mean squares for total oil, crude protein, and N concentration in 2018 in Fargo and plant height at harvest, seed oil, crude protein, and N concentration in two environments (Env) in 2019.

| | | 2018 | | | | 2019 | | |
| Sources of variation | df | Total oil | Seed crude protein | Seed N content | df | Seed oil | Seed crude protein | Seed N content |
|---|---|---|---|---|---|---|---|---|
| Env | | | | | 1 | 3137* | 197* | 5* |
| Rep (Env) | 6 | 404 | 17 | 0.45 | 6 | 651 | 158* | 4* |
| Sowing date | 3 | 447 | 58 | 1.50 | 3 | 1256 | 101 | 3 |
| Sowing date x env | - | | | | 3 | 880 | 68 | 2 |
| Error | 10 | 482 | 43 | 1.11 | 10 | 435 | 32 | 1 |
| CV, % | | | 8 | 2 | 2.09 | | 7 | 2 | 2 |

* Significant at 0.05 probability

Table 28. Analysis of variance and mean squares for fatty acids (16:0, 18:1, 18:2, 18:3, 20:1) in 2018 in Fargo and fatty acids (16:0, 18:1, 18:2, 18:3, 20:1) in two environments (Env) 2019.

| | | 2018 | | | | | | | 2019 | | | | | |
| Sources of variation | df | 16:0 | 18:1 | 18:2 | 18:3 | 20:1 | 22:1 | df | 16:0 | 18:1 | 18:2 | 18:3 | 20:1 | 22:1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Env | | | | | | | | 1 | 1.49* | 0.55 | 0.33 | 0.03 | 1.01* | 0.16* |
| Rep (Env) | 3 | 0.11* | 0.49 | 0.85 | 1.65 | 0.21 | 0.01 | 6 | 0.15* | 0.90* | 4.56* | 1.05* | 0.86* | 0.07* |
| Sowing date | 2 | 0.06* | 4.72* | 1.29 | 0.17 | 0.09 | 0.01 | 3 | 0.50 | 0.69 | 0.16 | 5.25 | 0.10 | 0.04 |
| Sowing date x env | | | | | | | | 3 | 0.36* | 0.40 | 0.03 | 4.90* | 0.05 | 0.05* |
| Error | 6 | 0.01 | 0.83 | 1.88 | 1.45 | 0.44 | 0.01 | 10 | 0.03 | 0.24 | 1.24 | 0.32 | 0.08 | 0.01 |
| CV, % | | 1.68 | 6.92 | 7.87 | 3.26 | 4.45 | 2.86 | | 2.93 | 3.37 | 6.39 | 1.60 | 1.73 | 2.42 |

* Significant at 0.05 probability.

Table 29. Mean content of total oil, crude protein and N concentration of camelina seeds in Fargo, ND, 2018.

| Sowing date | Total oil | Crude protein | N |
|---|---|---|---|
| | --------------------------------g kg$^{-1}$-------------------------------- | | |
| Date 1 | - | - | - |
| Date 2 | - | - | - |
| Date 3[a] | - | - | - |
| Date 4 | 263 | 307 | 50 |
| Date 5 | 267 | 315 | 50 |
| Date 6 | 247 | 313 | 50 |
| LSD (0.05) | NS[b] | NS | NS |

For all dates *n*=4.

[a] Sowing date 3 was skipped due to excessive moisture

[b] not significant

Table 30. Mean camelina seed oil, crude protein and N concentration averaged across two environments, Fargo and Prosper, ND, in 2019.

| Sowing date | Total oil | Crude protein | N |
|---|---|---|---|
| | --------------------------------g kg$^{-1}$-------------------------------- | | |
| Date 1 | - | - | - |
| Date 2 | 287 | 290 | 47 |
| Date 3 | 294 | 287 | 46 |
| Date 4 | 268 | 284 | 46 |
| Date 5 | 273 | 283 | 46 |
| Date 6 | - | - | - |
| LSD (0.05) | NS[a] | NS | NS |

For all values sowing date 2, and 5, *n*=2 and *n*=6 respectively. All other dates *n*=8.

[a] not significant

Table 31. Mean content of main fatty acids (% of total oil) from one environment in Fargo, ND, in 2018.

| Sowing date | Palmitic acid (16:0) | Oleic acid (18:1) | Linoleic acid (18:2) | Linolenic acid (18:3) | Eicosenoic acid (20:1) | Erucic acid (22:1) |
|---|---|---|---|---|---|---|
| | ---------------------------------------------------- % ---------------------------------------------------- | | | | | |
| Date 1 | - | - | - | - | - | - |
| Date 2 | - | - | - | - | - | - |
| Date 3[a] | - | - | - | - | - | - |
| Date 4 | 6.6 | 12.1 | 18.1 | 36.7 | 14.8 | 3.8 |
| Date 5 | 6.3 | 13.2 | 17.2 | 37.1 | 15.0 | 3.8 |
| Date 6 | 6.3 | 14.2 | 17.0 | 36.9 | 14.7 | 3.9 |
| LSD (0.05) | 0.2 | 1.6 | NS[b] | NS | NS | NS |

For all dates *n*=4.
[a] Sowing date three was skipped due to excessive moisture
[b] not significant

Table 32. Mean main fatty acids (% of total oil) averaged across two environments in Fargo and Prosper, ND, in 2019.

| Sowing date | Palmitic acid (16:0) | Oleic acid (18:1) | Linoleic acid (18:2) | Linolenic acid (18:3) | Eicosenoic acid (20:1) | Erucic acid (22:1) |
|---|---|---|---|---|---|---|
| | ---------------------------------------------------- % ---------------------------------------------------- | | | | | |
| Date 1 | - | - | - | - | - | - |
| Date 2 | 5.7 | 14.1 | 17.3 | 35.4 | 16.8 | 3.9 |
| Date 3 | 5.7 | 14.2 | 17.4 | 36.3 | 16.7 | 3.9 |
| Date 4 | 5.8 | 14.9 | 17.4 | 34.9 | 16.9 | 3.8 |
| Date 5 | 6.2 | 14.5 | 17.5 | 34.3 | 16.8 | 3.8 |
| Date 6 | - | - | - | - | - | - |
| LSD (0.05) | NS[a] | NS | NS | NS | NS | NS |

For all values sowing date 2, and 5, *n*=2 and *n*=6 respectively. All other dates *n*=8.
[a] not significant

# CHAPTER 4. SEEDLING MORPHOLOGY[1]

4.1. Abstract

Camelina [*Camelina sativa* (L.) Crantz] has two distinctive biotypes, summer and winter, with winter biotypes requiring vernalization to enter the reproductive phase. Increased interest in broadening the diversity of winter-hardy cover crops in the northern Great Plains of the U.S. to reduce soil erosion and nitrate leaching through the winter months had led seed companies to offer winter camelina seed outsourced from several other states. Regrettably, in 2017, all outsourced camelina seed from other states turned out to be summer biotypes that did not survive the North Dakota winter. The objective of this study was to determine the morphological characteristics of seedlings from summer- and winter-biotypes. Morphological characteristics of seedlings were determined by initially growing fifteen summer- and fifteen winter-biotypes in an environmental chamber. However, three accessions originally classified as the winter biotype bolted without vernalization and thus were treated as summer-biotypes. Morphological measurements were taken every week for four weeks. Significant interactions were present and observed for pairs of vegetative leaves, growth stage, height, leaf length, leaf width and the number of lobes. Pairs of vegetative leaves at 21 days after emergence (DAE) was 1.7 pairs of leaves. While at 35 DAE plant height ranged from 1-mm from winter types to 562-mm from summer types. Differences in seedling morphological characteristics can be used to differentiate winter- or summer-biotypes.

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[1] Note: The information presented in this chapter has been published in:

## 4.2. Introduction

### 4.2.1. Seedling Morphology

Initially, 15 summer and 15 winter accessions, including both cultivars and breeding lines, were selected to determine the morphological differences between summer- and winter-camelina biotypes. Selected accessions were chosen from a collection of winter- and summer-biotypes of camelina obtained from researchers in Minnesota, Kansas, Montana, Austria, and the USDA germplasm collection in Ames, IA (http://www.ars-grin.gov/npgs/). Four accessions originally classified as winter biotypes bolted and formed inflorescences without vernalization and were treated as summer biotypes, which resulted in a final number of 19 summer and 11 winter accessions (Table 33).

Morphological characterization of the selected accessions was carried out at the Edward T. Schafer Agricultural Research Center, Fargo, ND, in a controlled environmental chamber. The accessions were evaluated in a randomized complete block design with three replicates, and it was repeated twice. Treatments were summer- and winter-biotype and date of measurement [11, 18, 25, and 32 days after emergence (DAE)]. The environmental chamber (Conviron, PGR15, Conviron, Winnipeg, Manitoba, Canada), was equipped with 4000 lumens fluorescent light bulbs (Philips #F72T8/TL841/HO Fluorescent, T8, R17d, 65 W), was set for a 16 h-light/8 h-dark period and a constant temperature of 22°C throughout the experiment. Accessions were planted into Sunshine mix #1 (Fisons Horticulture Inc., Bellevue, WA, USA) in Ray Leach cones (SC10 Super: 3.6 cm × 20.9 cm, 164 mL volume; Stuewe & Sons, Inc., Tangent, OR, USA) and placed in supporting trays. After emergence, plants were thinned to two plants per cone and two weeks after sowing they were thinned again to one plant per cone. Leaf measurements were taken on the top two leaves that were fully developed. Measurements were taken every week, starting two weeks after sowing and every week thereafter for three weeks (Table 34).

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Table 33. Accession name, detailed know origin and source of the camelina seed accessions used for morphology classification.

| No. | Biotype | Accession | Origin |
|---|---|---|---|
| 17 | Summer | CK2X-7 | Austria |
| 26 | Summer | CJ10X-13 | Austria |
| 61 | Summer | CJ11X-96 | Austrian |
| 65 | Summer | CJ11X-100 | Austrian |
| 71 | Summer | CJ11X-104 | Austrian |
| 74 | Summer | CK1X-110 | Austrian |
| 75 | Summer | CK2X-110 | Austrian |
| 90 | Summer | CJ7X-121 | Austrian |
| 146 | Summer | CA9X-8 | Austrian |
| 189 | Summer | PI 304269 | Sweden |
| 201 | Winter | PI 650143 | Germany |
| 222 | Summer | PI 650164 | Austria |
| 229 | Winter | Joelle | USDA |
| 230 | Winter | Bison | Colorado |
| 231 | Winter | BSX-WG1 | Colorado |
| 234 | Winter | Luna | Poland |
| 235 | Winter | Maczuga | Poland |
| 236 | Winter | PI 311736 | Poland |
| 239 | Winter | PI 650155 | Poland |
| 241 | Winter | PI 650158 | Poland |
| 242 | Summer | PI 650163 | USDA |
| 246 | Winter | CN 113660 | Poland |
| 247 | Winter | CN 113691 | Germany |
| 253 | Summer | CN 113668 | Canada |
| 262 | Summer | Ligena | Montana |
| 263 | Summer | Suneson | Montana |
| 268 | Summer | Blaine Creek | Kansas |
| 273 | Summer | Pronghorn | Kansas |
| 275 | Summer | Shoshone | Kansas |
| 279 | Summer | CO-46 | USDA |

Table 34. Planting, emergence and measurement dates for each run in 2018.

| | Run 1 | Run 2 |
|---|---|---|
| Planting date | 6 March | 20 November |
| Emergence | 9 March | 23 November |
| Sampling date 1 | 20 March | 4 December |
| Sampling date 2 | 27 March | 11 December |
| Sampling date 3 | 3 April | 18 December |
| Sampling date 4 | 10 April | 26 December |

Accessions were evaluated on the top two fully-developed leaves (number of lobes, width and length of the blade, petiole, and overall length), plant height, pairs of leaves, and stage (cotyledon, vegetative, budding, and flowering). Stages were assigned numbers [vegetative (1) "101-309 BBCH", budding (2) "500-501 BBCH", flowering (3) "600-609 BBCH", and cotyledons (4) "100"]. Plant height measurements and a visual trichrome rating were taken at both runs, while a visual lobe depth rating was also taken during the second run. Quantitative traits of leaves in the first run and the first sample date of the second run were first measured with a manual caliper, and thereafter were measured with a digital caliper. Plant height measurements were taken with a ruler.

*4.2.2. Statistical Analysis*

Statistical analysis was conducted using SAS 9.4 software, procedure MIXED (S.A.S. Institute, 2014), using standard procedures for a randomized complete-block design. Trait error means squares were compared for homogeneity between runs according to the fold $F$-test, and if homogeneous, a combined ANOVA was performed. Runs and accessions were fixed effects.

4.3. Results and Discussion

The combined analysis of variance across runs had significant interaction ($P \leq 0.05$) between biotype and measurement date for seven different characteristics including: pairs of fully developed vegetative leaves, growth stage, plant height, leaf length of the first most developed leaf, width of the first most developed leaf, and the number of lobes on the first most developed leaf. The rest of the characteristics did not have significant interaction between biotype and measurement date (Table 35 and 36).

*4.3.1. Leaf Number, Growth Stage, and Plant Height*

Pairs of leaves mean ranged between 0.85 and 8.64 across measurement dates (Fig. 4a). Pairs of leaves mean across replicates and runs were similar between the biotypes up until 14 DAE when summer-biotype accessions started to develop more leaves than winter-biotypes as many of the summer accessions started to bolt. At 21 DAE, the difference between summer- and winter-biotype was 1.7 pairs of leaves. Over a period of 30 days of growth, Passardi et al. (2006) demonstrated that *Arabidopsis thaliana* continued to develop leaves. However, near the last measurement dates several of the different *Arabidopsis* biotypes used did not continue to develop more leaves, and the total number was lower then what was observed in camelina in this study.

The growth stage rating of plants ranged from 1 to 2.54 across the different DAE (Fig. 4b). The large difference at 35 DAE can be attributed to the summer-biotype accessions entering the reproductive phase, while the winter-biotype accessions stayed in the vegetative stage, as they require a vernalization period to induce flowering.

Table 35. Analysis of variance and mean squares for leaf measurements of two camelina biotypes and four days after emergence (DAE).

| Sources of variation | df | Total leaf length (second) | Leaf width (second) | df | Blade length (second) | Petiole length (second) | df | Total leaf length (first) | Leaf width (first) | df | Blade length (first) | Petiole (first) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Run | 1 | 5369 | 2838* | 1 | 44.2 | 110.92* | 1 | 22228* | 2023* | 1 | 724* | 1 |
| Rep (Run) | 2 | 27345 | 30 | 2 | 12.6 | 77.98* | 2 | 261 | 23 | 2 | 654* | 678* |
| Biotype | 1 | 9406 | 760* | 1 | 55.0 | 50.10 | 1 | 172 | 476* | 1 | 378* | 27 |
| DAE | 3 | 183031* | 6373* | 2 | 32196.5* | 843.16* | 3 | 34678* | 2131* | 2 | 5268* | 92* |
| Biotype x DAE | 3 | 2294 | 33* | 2 | 85.9 | 19.36 | 3 | 4755* | 234* | 1 | 30 | 59 |
| Run x DAE | 3 | 8903 | 1126* | 2 | 542.9* | 268.69* | 3 | 9677* | 678* | 2 | 642* | 216* |
| Run x biotype | 1 | 19884 | 61* | 1 | 0.4 | 3.11 | 1 | 643* | 13 | 1 | 8 | 10 |
| Run x biotype x DAE | 3 | 18739 | 9 | 1 | 0.8 | 0.39 | 3 | 247 | 17 | 1 | 78 | 29 |
| Error | 700 | 24686 | 10 | 369 | 50.2 | 22.78 | 693 | 113 | 12 | 239 | 70 | 30 |
| CV, % | | 260 | 19 | | 24.9 | 65.89 | | 19 | 21 | | 25 | 64 |

* Significant at 0.05 probability. Experiment was conducted twice (runs).
First leaf corresponds to the upper most develop leaf, second leaf corresponds to the leaf below the upper developed leaf.

Table 36. Analysis of variance and means squares for pairs of vegetative leaves, growth stage, plant height, leaf lobes, trichome rating, and lobe degree of two biotypes and four days after emergence (DAE).

| Sources of variation | df | Pairs of vegetative leaves | Growth stage | Plant height | df | Lobes (second) | df | Lobes (first) | df | Trichome | df | Lobe degree |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Run | 1 | 29.09* | 0.43 | 13248* | 1 | 1.08 | 1 | 18.94* | 1 | 10.20* | - | - |
| Rep (Run) | 2 | 1.52 | 0.16 | 4370 | 2 | 1.06 | 2 | 0.49 | 2 | 0.06 | 2 | 0.48 |
| Biotype | 1 | 69.71* | 16.42* | 1772253* | 1 | 0.01 | 1 | 1.85 | 1 | 116.76* | 1 | 98.64* |
| DAE | 3 | 928.75* | 19.25* | 748173* | 1 | 1.41 | 2 | 19.85* | 1 | 0.95 | 1 | 0.50 |
| Biotype x DAE | 3 | 24.17* | 24.05* | 778286* | 1 | 4.09 | 2 | 15.62* | 1 | 0.02 | 1 | 0.90 |
| Run x DAE | 3 | 93.48* | 0.91* | 11852* | 1 | 2.72 | 2 | 4.24 | 1 | 3.22* | - | - |
| Run x biotype | 1 | 1.20 | 1.23* | 10438* | 1 | 6.30 | 1 | 0.21 | 1 | 0.95* | - | - |
| Run x biotype x DAE | 3 | 2.64* | 0.31 | 12454* | 1 | 2.17 | - | - | 1 | 0.02 | - | - |
| Error | 702 | 0.53 | 0.17 | 2416 | 165 | 4.85 | 217 | 3.25 | 350 | 0.21 | 126 | 0.33 |
| CV, % | | 18.74 | 32.63 | 70 | | 43.43 | | 30.32 | | 23.94 | | 23.16 |

* Significant at 0.05 probability. Experiment was conducted twice (runs).
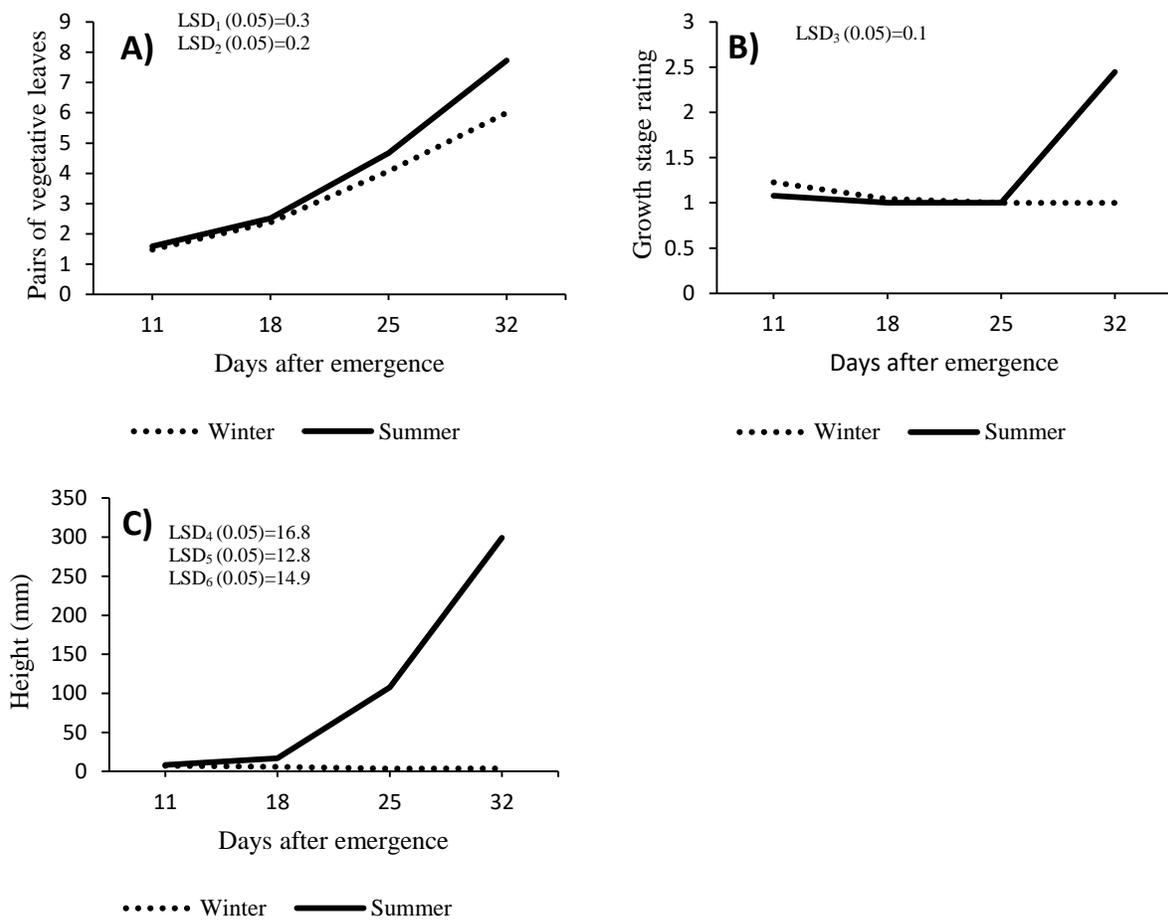
Fig. 4. Weekly measurement for A) pairs of vegetative leaves, B) growth stage rating (1-4), and C) plant height of summer and winter camelina biotypes averaged across two runs. $LSD_1$ to compare means between days after emergence (DAE) for a same biotype. $LSD_2$ to compare means of biotypes for a same or different DAE. $LSD_3$ to compare between means across and between biotypes and DAE. $LSD_4$ to compare means between DAE for a same biotype. $LSD_5$ to compare means of biotypes for a same DAE. $LSD_6$ to compare means of biotypes in different DAE.

Plant height ranged between 1-mm from winter-biotypes at 35 DAE to 562-mm from summer-biotypes at 35 DAE (Fig. 4c). The large difference between 21 and 35 DAE for summer-biotypes can also be attributed to summer-biotypes bolting and entering reproduction while winter-biotypes remained vegetative. Plant height measurement for both summer- and winter-biotypes from this study are considerably shorter than what was reported by Anderson et

al. (2018). This difference could possibly be attributed to the larger size containers used by Anderson et al. (2018) or that they evaluated only one summer- and one winter-biotype.

*4.3.2. Total Leaf Length and Width*

Total leaf length of the upper most developed leaf increased for both biotypes until 28 DAE when the winter-biotype stopped growing and the summer biotype new leaves were shorter by 15 mm (Fig. 5a). The cause for the decrease in summer-biotypes was that the new fully developed leaves were considerably smaller and shorter than leaves that emerged earlier. Winter and summer-biotypes were different in leaf length at every date except at 21 DAE. Winter biotype leaves were 13.8-mm longer at 35 DAE than the summer-biotype leaves.

Leaf width of the upper most developed leaf was different at 21, 28, and 35 DAE ($P \leq 0.05$) (Fig. 5b). In summer-biotype accessions leaf width increased between 14 and 21 DAE and decreased thereafter, while in winter biotype accessions, leaf width increased until 28 DAE and leveled off thereafter.

Total leaf length of the second most developed leaf averaged across winter- and summer-biotypes increased from 14 to 35 DAE for a maximum length of 96.4 mm at 35 DAE (Fig. 5c). The second most developed leaves were longer for either winter- or summer-biotypes than the first most developed leaf at 28 and 35 DAE. This could be attributed to the fact that leaves already developed continued to grow in length as new leaves were developed in the apical meristem.

Leaf width of the second most developed leaf did not vary greatly until 35 DAE, where a considerable difference was observed between the two biotypes (Fig. 5d). At 35 DAE, leaf width of winter-biotypes averaged 24 mm while summer-biotypes only averaged 8 mm in width. For winter-biotypes, leaf width increased until 28 DAE leveling off thereafter, which follows the

trend of leaf width of the first most developed leaf. Hopkins et al. (2008) demonstrated a correlation between lower latitude ecotypes of *A. thaliana* and elongated petioles along with increased total leaf length. This could possibly explain variation between accessions and between winter- and summer-biotypes, however, we do not have accessions from lower latitudes to prove this.  Leaf length and width in *A. thaliana* is controlled by two regulatory genes, *AN3* and *AtGRF5,* that encode a putative transcription co-activator and putative transcription factor, respectively (Horiguchi et al., 2005). It is likely that the genes controlling leaf length and width in *C. sativa* are the same or similar to *A. thaliana,* which could possibly explain the observed differences on leaf length and width between biotypes in this study.  However, further research on the transcript abundance and activity of these proteins would be needed to confirm this hypothesis.

*4.3.3. Blade and Petiole Length*

Blade length of the upper most developed leaf averaged across biotypes ranged from 28 and 52 mm, for 14 and 28 DAE, respectively (Fig. 6a).  Blade length of the upper most developed leaf continued to elongate until 35 DAE but at a slower growth rate than the blade length of the second most developed leaf (Fig. 6b). Blade length increase between 14 and 28 DAE was only 25 mm, which is considerably lower than the increase of 43 mm from second most developed leaf.

Petiole length of the first most developed leaf averaged across biotypes was shorter from 14 to 28 DAE by 2.9 mm (Fig. 6c). This decrease can be attributed to the increase in blade length of newly developed leaves (Fig. 4a), while the total leaf length leveled off for the winter-biotype at 28 DAE (Fig. 5a).

Petiole length of the second most developed leaf, averaged across both winter- and summer-biotype accessions, increased from 14 to 21 DAE leveling off between 21 and 28 DAE (Fig. 6d). Petiole growth stopped after 21 DAE, which can be attributed to the large increase in blade length of the second upper most developed leaf. At 35 DAE, petioles were no longer observed on either of the two biotypes, meaning leaves thereafter were sessile. Blade and petiole length were no longer evaluated at 35 DAE as leaves became sessile and total leaf length discussed earlier represented both.
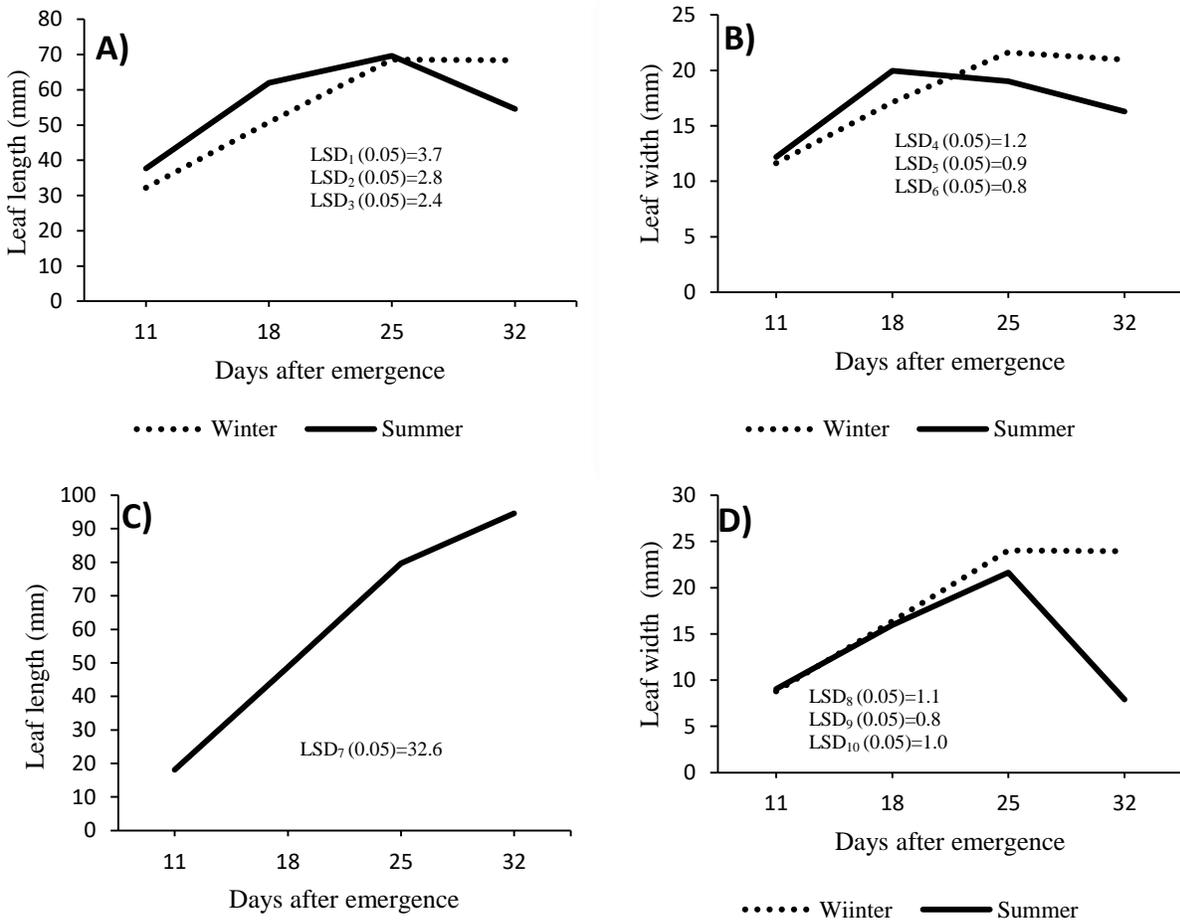
Fig. 5. Winter and summer-biotypes leaf morphology A) total leaf length of the upper most developed leaf, B) upper most developed leaf width, C) total leaf length of the second most upper developed leaf averaged across both biotypes, and D) second most upper developed leaf width. $LSD_1$, $LSD_4$, $LSD_8$ to compare means between dates for a same biotype. $LSD_2$, $LSD_5$, $LSD_9$ to compare means of biotypes for a same date. $LSD_7$ to compare means among dates averaged across both biotypes. $LSD_3$, $LSD_6$, $LSD_{10}$ to compare means of biotypes and in different dates.

In *A. thaliana*, ratio changes between total leaf length and blade length were affected by increased length of total leaf length (Hopkins et al., 2008). The ratio was affected by changes in the petiole length rather than the blade length on the leaves, which was the opposite of what was observed in this study in camelina. While differences in visual appearance between the leaf blade and petiole are observed, formation of the leaf blade and the leaf petiole occurs at the same proliferative zone of the leaf primordia and no clear boundary existed between the two in *A.*

*thaliana* (Ichihashi et al., 2011). At maturation of the leaf primordia the proliferative region was no longer maintained, thus no longer supporting cell division of blade or petiole cells (Ichihashi et al., 2011).
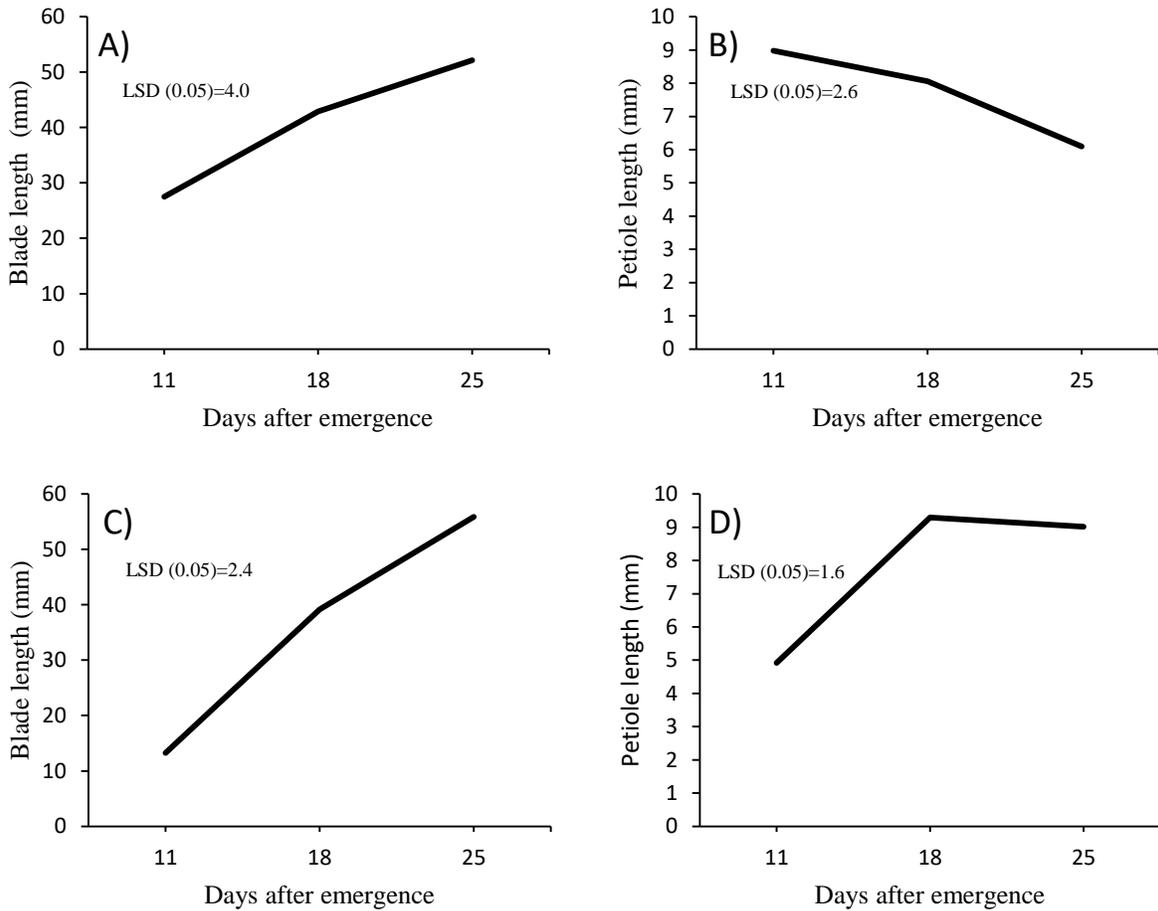


Fig. 6. Leaf morphology of camelina averaged across biotypes: A) blade length of the upper most developed leaf; B) blade length of the second most upper most developed leaf; C) petiole length of the upper most developed leaf; and D) petiole length of the second most upper developed leaf. LSDs in each figure are to compare means among days after emergence averaged across both biotypes.

### 4.3.4. Trichome Rating, Number of Lobes, and Lobe Degree Rating

A visual trichome rating was taken at 28 and 35 DAE. A significant ($P \leq 0.05$) trichome rating averaged across biotypes was observed between both DAE (Fig. 7a). Winter-biotypes had a significantly higher rating than summer camelina indicating that as plants developed, they had

less visible trichomes. In *A. thaliana*, Passardi et al. (2006) reported that inbred line Wassiewskija had a higher number of trichomes on leaves and the number of branches a trichome had varies between different inbred lines.

Lobe degree rating was only taken on the second run and only on the accessions that had lobes on the top two most developed leaves at 28 and 35 DAE. From 28 to 35 DAE, the average rating increased 0.06 averaged across both biotypes (Fig. 7b). While the average lobe degree rating in the winter accession was relatively stable at about 3.4 at both 28 and 35 DAE, the average of summer accessions increased slightly from 1.5 to 1.8 at 28 and 35 DAE, respectively, which was significant between biotypes at both measurement dates but not by interaction between biotype and measurement dates. Inhibition of growth at the sinuses of leaf lobes is controlled by three *HD-ZIP* transcription factors encoded by the *REDUCED COMPLEXITY* locus (Fritz et al., 2018). This locus has been through two successive gene duplication events followed by a functional divergence in which each copy of the gene can regulate growth in different areas of the leaves. Changes in the functional regulation of the gene could cause the observed differences between the summer and winter biotypes. In *Brassica napus* L., a homeobox gene, *BnA10.LMI1*, regulates the development of lobes and cis-regulatory divergences caused by different allele effects (Hu et al., 2018).

Total number of lobes on the upper most developed leaf did not vary to a large degree between any of the measurement dates or between summer- and winter-biotypes, however, there were significant differences between winter- and summer-biotypes at 28 and 35 DAE (Fig. 7c). The number of lobes on the second most developed leaf rose from 4.6 to 5.3, averaged across biotypes between 28 and 35 DAE, respectively (Fig. 7d).

A)

Visual trichome rating

2
1.98
1.96
1.94
1.92
1.9
1.88
1.86
1.84
1.82

LSD$_1$ (0.05)=2.0

25    32

Days after emergence

B)

Visual lobe degree rating

2.52
2.5
2.48
2.46
2.44
2.42
2.4

LSD$_2$ (0.05)=0.2

25    32

Days after emergence

C)

Number of lobes

7
6
5
4
3
2
1
0

LSD$_3$ (0.05)=1.1
LSD$_4$ (0.05)=3.3

11    18    25

■Winter ▨Summer

Days after emergence

D)

Number of lobes (mm)

5.4
5.2
5
4.8
4.6
4.4
4.2

LSD$_5$ (0.05)=0.7

25    32

Days after emergence
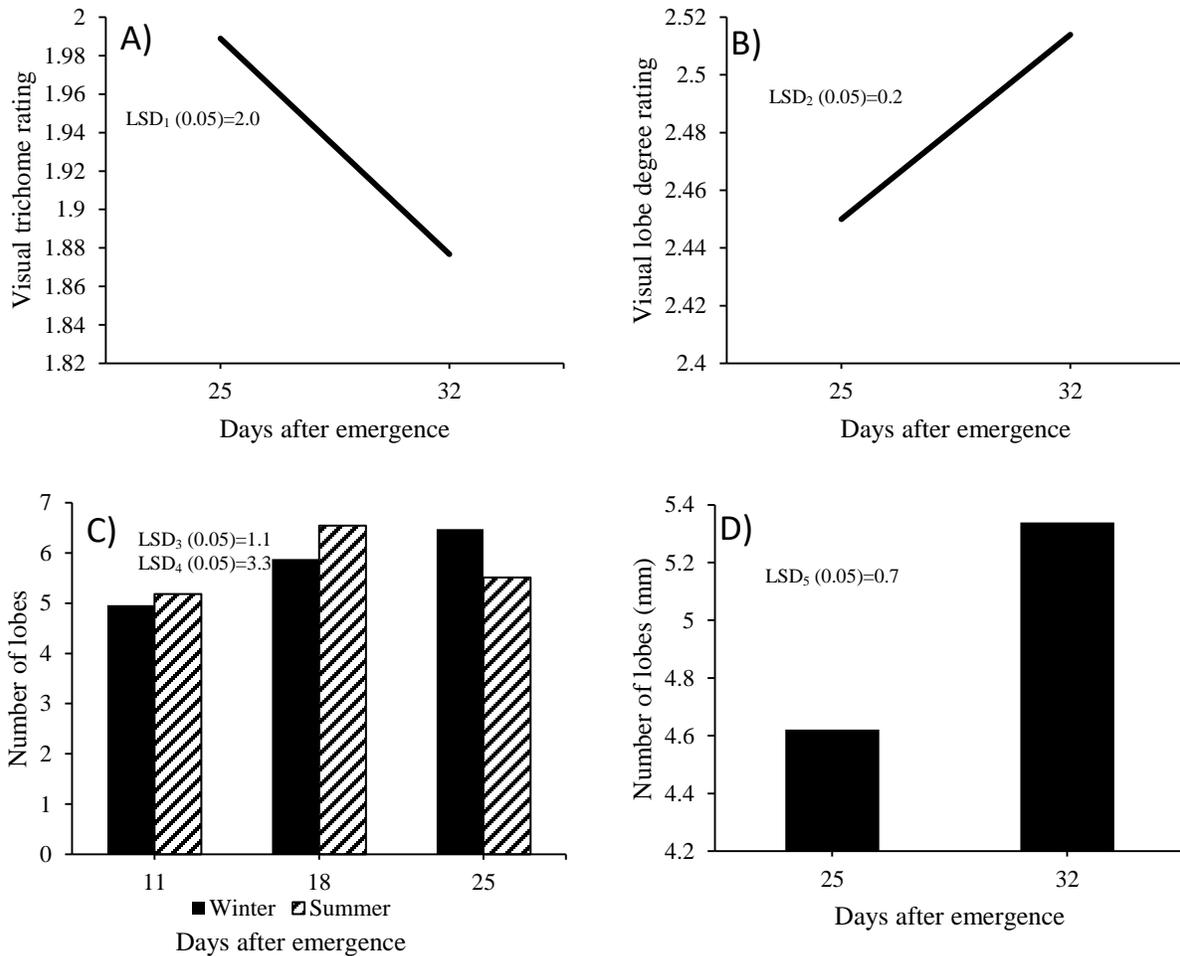
Fig. 7. Plant trichome and leaf lobe morphology A) whole plant trichome rating (rating 1, limited trichomes and 3, large number of trichomes); B) lobing degree (rating 1, small lobes and 5, large lobes); C) number of lobes on the upper most upper developed leaf for each biotype; and D) number of lobes on the second most developed leaf. LSD$_1$, LSD$_2$, and LSD$_5$ in each figure are to compare means among days after emergence (DAE) averaged across both biotypes. LSD$_3$ to compare means between DAE for a same biotype. LSD$_4$ to compare means of biotypes for same DAE.

4.4. Conclusion

Winter- and summer-biotypes of camelina evaluated in this study had some distinct morphological phenotypes. Significant differences were observed for pairs of vegetative leaves, growth stage, height, total length, width, and number of lobes of the upper most developed leaf, and width of the second most upper leaf between winter- and summer biotypes. These morphological differences can allow both researchers conducting experiments under controlled

conditions to determine the correct biotype of unknown seed or producers sowing a questionable

seed lot when conditions are favorable for adequate growth.

# CHAPTER 5. SEED CHARACTERIZATION AND REFLECTANCE PROPERTIES[2]

## 5.1. Abstract

Camelina [*Camelina sativa* (L.) Crantz] has two distinctive biotypes, summer and winter, with winter biotypes requiring a vernalization treatment to enter the reproductive phase. Increased producer interest in broadening the diversity of winter-hardy cover crops in the northern Great Plains of the U.S. to reduce soil erosion and nitrate leaching through the winter months had led seed companies to offer winter camelina seed outsourced from several other states. Regrettably, in 2017, all outsourced camelina seed from other states turned out to be summer biotypes that did not survive the North Dakota winter. The objective of this study was to determine differences in seed wavelength absorbance between winter- and summer-biotypes both visible and near-infrared spectra were examined, which encompass 400 to 2498 nm wavelengths. Mixtures of cultivars Joelle (winter) and one summer type were analyzed using a near infrared spectroscopy (NIRS), XDS Analyzer; seed mixtures were prepared in increments of 5% of 'Joelle'. Mixtures of different lots of 'Joelle' were prepared in increments of 25%. Spectrum of scanned field grown seed lots were used to develop the calibration equations. The equation developed to predict the ratio of winter seed in a seed lot performed very well ($r^2$=0.96), being

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able to distinguish known field grown biotypes used in the equation. Having a rapid method to determine the percentage of winter camelina in an unknown seed sample will be very favorable for all producers, as well as seed companies, interested in growing winter camelina either as a winter-hardy cover crop or as a winter annual cash crop.

5.2. Introduction

Field-grown seed samples of known winter- and summer-biotypes were obtained from researchers in Minnesota, Kansas, Montana, Wyoming, and North Dakota. Summer cultivars included Blaine Creek, Pronghorn, Shoshone, Suneson, and Ligena. Winter cultivars included Joelle, Bison, and BSX-WG1.

Seeds from one winter-biotype and one summer-biotype were bulked in known proportions (increments of 5%, from zero to 100%). Mixtures of cultivars Joelle, Blaine Creek, Shoshone, and Pronghorn were bulked and scanned in duplicates. Five mixtures of two different 'Joelle' seed samples from different locations and years were bulked in known proportions (increments of 5%, from zero to 100) and then scanned in duplicates. A total of 119 different seed mixes combinations and pure cultivars were scanned.

Sample scanning was conducted with a Foss XDS near Infrared instrument (XDS analyzer, Foss, Denmark) over a wavelength spectrum of 400-2498 nm. Crystal ring cups with an outside diameter of 5.1 cm and an inside diameter of 3.8 cm were filled with 1.8 g of whole seeds and a disposable back foam board was placed on the back to ensure an even coverage of seed across the crystal before scanning. Spectrum data was exported from Mosaic version 8.4.4.15 at spectrum resolution of 2 nm. Near infrared spectra analyzed with WinISI version 4.10.015326 software was used to develop a calibration equation for percent winter-biotype seed in a seed sample. After the spectral data were imported into WinISI, the duplicates were averaged, and the

averages were then used to develop the calibration equations. Data consisting of the percent

winter-biotype from each of the mixes and the known winter and summer cultivars were

imported into the WinISI and used with the NIR spectrum data to develop a calibration file.

Eight different pre-spectra processing methods were used for scatter correction (Table 37). After

pre-spectra processing was complete, the same methods were used to develop the calibrations

from each of the pre-spectra processing results. Sixty-four different equations were tested. Along

with the different math treatments, modified partial least squares (PLS) regression and four

groups of cross validation were used to develop calibration equations. Calibration equation

models were compared by standard error of cross validation (SECV) and cross validation (1-VR)

values, and evaluated by the coefficient of determination ($r^2$). Additional validation of the near-

infrared spectroscopy (NIRS) equation was carried out by only running samples of known

biotypes. A paired *t*-test was used to determine if the developed calibration could distinguish

between summer and winter-biotype accessions.

Table 37. Scatter correction and math treatments used for both pre-spectra processing and
mathematical treatments.

| Scatter | Math treatment |
|---|---|
| SNV and detrend[a] | 1,4,4,1 |
| SNV and detrend | 2,4,4,1 |
| None | 1,4,4,1 |
| None | 2,4,4,1 |
| SNV and detrend | 2,6,4,1 |
| SNV and detrend | 2,6,6,1 |
| SNV and detrend | 3,5,5,1 |
| SNV and detrend | 1,16,16,1 |

[a] standard normal variate

5.3. Results and Discussion

The calibration settings for the final selected equation were pre-spectra processing

treatment of standard normal variate (SNV) and detrend scatter correction 3,5,5,1. The

calibration settings were math treatment 2,6,4,1 SNV and detrend scatter correction, both with

two passes of outlier elimination. The calibration equation had an $r^2$ of 0.96, a standard error of

calibration (SEC) of 2.58%, and the ratios of performance to deviation (RPD) was 7.84 (Fig. 8).
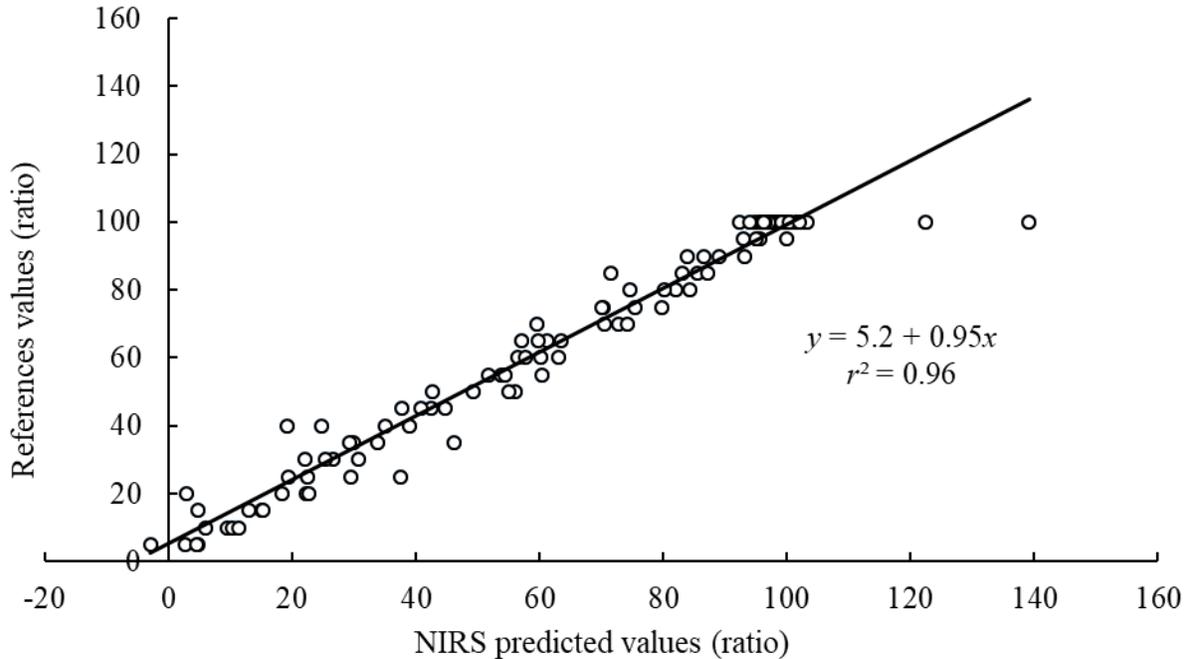


Fig. 8. Predicted vs reference values for seed reflectance using near-infrared spectroscopy (NIRS).

The predicted values against the reference values and the RPD value indicated a high

prediction ability for the NIRS equation to determine the ratio of winter-biotype seed in an

unknown seed lot. Some outliers had predicted values much higher than reference values of

known seed lots of winter camelina seed. This could be attributed to the age of the seed lots and

the limited number of winter cultivars used to build the equations. Differences in the

pigmentation of the seed coat have also previously been noticed between winter- and summer-

biotypes of camelina (Wiwart et al., 2019). Differences in *A. thaliana* seed coat pigmentation is

due to the accumulation of anthocyanins in the testa and tannin precursors in the endothelium

(Passardi et al., 2006). Seed age may also change the reflectance properties of seed, which has

been observed in seeds of soybean as well as Asian rice (*Oryza sativa* L.) (Li et al., 2008; Bazoni et al., 2017). Soybean seeds stored at 35°C exhibited physicochemical alterations in pH and free fatty acids and increased temperature during storage led to faster color degradation (Bazoni et al., 2017).

The predictability of the developed NIRS calibration was tested by running spectra data of previously scanned camelina seed samples against the developed equation. A total of 37 samples constituting of 22 winter and 15 summer accessions were scanned including released cultivars and breeding lines produced under controlled and field conditions. When evaluating the ratio of winter seed among the different accessions, a great number of the summer accessions had high ratio values up to 95. Winter accessions also had a large variation, ranging from 71 to 152. When determining the cause of these wide ranges, we noticed that many of the samples giving varying results were not included in the construction of the equation or were from a different cultivar than what was used to build the calibration. Several of the seed samples also appeared to possibly be harvested when the seed was not fully developed, which may change the reflectance properties of the seed. We assumed that some of these seeds were harvested before physiological maturity, full maturity defined by Martinelli and Galasso (2011) is when siliques are ripe for harvest, corresponding to BBCH code of 809. Physiological maturity in camelina occurs prior to full plant ripening and after than maximum seed yield and oil concentration (Walia et al., 2018). Some seed lots were several years old and records related to their storage were lacking. Previous work by Walia et al. (2018) demonstrated that harvest timing and GDD accumulation influences seed yield, protein, and oil concentration, and the composition of fatty acids changed as seeds developed and matured. Seed growth and development under different field environments has also previously been shown to influence seed protein and oil

concentration of camelina (Berti et al., 2011; Gesch et al., 2018). These factors could possibly

also influence the ability of NIRS to determine the ratio of winter-biotypes. Further evaluation

was carried out by only running samples of known field grown winter- and summer- biotypes.

That resulted in a considerably narrower range of values for both the winter and summer

accessions. New ranges of seed ratios were -17 to 7and 96 to 104 for summer and winter

accessions, respectively. A paired *t*-test at 0.05 significance was performed to confirm if the

calibration could distinguish between summer- and winter- biotype cultivars used to develop the

equation and those were grown under field conditions and harvested after physiological maturity.

5.4. Conclusion

The calibration equation obtained with NIRS for identifying unknown or mixed seed lots

of camelina seed proved good performance for field grown seed lot samples. The calibration is

extremely useful for determining if a seed lot is a winter- or summer-biotype before seed is sent

to producers for planting, and at a fraction of the time and expense of growing out the seeds to

determine the biotype.

# CHAPTER 6. NEAR-INFRARED CALIBRATION OF CAMELINA SEEDS FOR CRUDE PROTEIN, TOTAL OIL, AND FATTY ACID PROFILE[1]

## 6.1. Abstract

Fast, non-destructive methods for determining the seed[3] composition of camelina [*Camelina sativa* (L.) Crantz] would be beneficial in evaluating germplasm for important agronomic traits.  In this study, near infrared spectroscopy (NIRS) methods were developed and evaluated for conducting non-destructive, high throughput phenotyping of seed quality traits. Crude protein and total oil concentration for 85 accessions (63 summer- and 22 winter-biotypes) were first determined by established wet chemistry methodology; whereas, for fatty acid profiles 173 accessions (149 summer- and 24 winter-biotypes) were determined using Gas Chromatography (GC). The wet chemistry and GC data were used to develop NIRS calibration equations for each trait. Based on the wet chemistry data obtained from 85 accessions, mean crude protein concentration was significantly less in summer (300 g kg$^{-1}$) than in winter (315 g kg$^{-1}$) biotypes ($P \leq 0.05$) and total oil was greater in seeds of summer (351 g kg$^{-1}$) than that of winter (326 g kg$^{-1}$) biotypes. Coefficient of determination for crude protein and oil concentration

($r^2$=0.98 and 0.89, respectively) and ratio of performance to deviation (RPD=9.2 and 4.3, respectively) indicated a high level of confidence for predicting these traits using NIRS. The most abundant fatty acid was linolenic acid (18:3) ranging from 22.8 to 38.4%, followed by linoleic acid (18:2) at 15.2-27.1%, eicosenoic acid (20:1) at 11.6-18.2%, and oleic acid (18:1) at 9.1-22.1%. Calibration models for the main fatty acids oleic, linoleic, linolenic, and eicosenoic acids had $r^2$ values of 0.72, 0.79, 0.83, and 0.56, respectively. All models were significant at 0.05 significance. Results of this study indicate that NIRS has potential as a non-destructive, high throughput method for determining quality traits of camelina seed.

## 6.2. Introduction

### 6.2.1. Plant Materials

Seed samples from 173 accessions of camelina were used in this experiment. Seed accession lots were obtained from USDA-ARS, Morris, MN; Univ. of Minnesota, St. Paul, MN; and North Dakota State University, Fargo, ND. The collection of existing seed lots from these 173 accessions were used for conducting wet chemistry and NIRS analyses, in most cases without further propagation. Furthermore, a study by Chao et al. (2019) indicated that some available accessions of camelina seed are likely misclassified for summer- and winter-annual biotype. To ensure that all 173 accessions used in this study were correctly classified for summer- and winter-biotype, a subset of seed from each accession was first phenotyped for bolting characteristics using standard greenhouse and environmental chamber conditions (Anderson et al., 2018; Chao et al., 2019). Based on the ability to bolt and flower without a vernalization treatment (8 weeks at 5°C), 24 of the 48 accessions previously designated as winter biotypes among the 173 accessions were re-classified as summer biotypes. Thus, within the 173 accessions of camelina used in this study, 149 were classified as summer biotypes and 24 were classified as winter biotypes. However, due to limited seed for some accessions within the

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collection, wet chemistry data for crude protein and total oil was obtained only from 85

accessions, whereas wet chemistry data for fatty acid profiles was obtained using all 173

accessions of camelina.

*6.2.2. Oil and Crude Protein Concentration*

The total oil concentration of seeds was determined as described in AOAC Official

Methods of Analysis 920.39. The nitrogen concentration of seeds was determined using a

Tecator Kjeltec 1030 Autoanalyzer and the modified Kjeldahl procedure as described by AOAC

Official Method 988.05. Crude protein concentration was calculated by multiplying N content by

6.25.

*6.2.3. Fatty Acid Profiling by GC Analysis*

In this study, the procedure of Vick et al. (2004) was modified and used for fatty acid

methyl esters (FAME) production. Approximately 60 mg of camelina seed was added to each

well (1 mL) of a 96-well plate (1896-1110, USA Scientific, Ocala, FL) containing a 4-mm

stainless steel bead and cap (1494-0400, USA Scientific). Seeds were disrupted with two 30-s

pulses (1800 rpm) on a bead beater (FastPrep96, MP Bio, Santa Ana, CA). A quick spin was

included to remove the tissue from the plate caps. A 450 µL volume of extraction solution (8 mL

methanol, 2 mL 0.5 N sodium methoxide in methanol [403067, Sigma-Aldrich, St. Louis, MO]

and 0.005 g BHT [butylated hydroxytoluene, PHR1117-1G, Sigma-Aldrich]) was added to each

well prior to shaking for 1 h at top speed (4625 Labline Titer Shaker, Thermo Scientific). A

quick spin was included to remove the extraction buffer from the plate caps. Then, 500 µL

hexane was added to each well, and wells were re-capped and vigorously shaken by hand. A

quick spin was included to pellet the tissue and separate the aqueous/hexane phases. Then, 250

µL of the hexane was transferred to a screw cap autosampler vial containing 500 µL hexane (750 µL final volume) for FAME analysis.

A gas chromatograph (Trace 1310, Thermo Scientific, Waltham, MA) with a flame-ionization detector and auto-sampler were used to measure fatty acids of camelina seed oil. Analyses were conducted with a 30-m × 0.25-mm DB-23 capillary column (Agilent Technologies, Inc., Santa Clara, CA) using the following parameters: He gas flow (1.9 mL min$^{-1}$ at 200 kPa) and injector and detector temperature (230 and 250°C, respectively). For each sample run, temperature started at 190°C for 4 min, then increased to 220°C (15°C min$^{-1}$) and was held at 220°C (1 min) prior to increasing the temperature to 240°C (25°C min$^{-1}$) and holding at 240°C (1 min). Standards 17A, 21A, 68B, and 411 (Nu-Chek-Prep, Inc., Elysian, MN) containing a mix of fatty acid methyl esters were used as references. The retention times of standards were used for identifying individual fatty acid peaks of each sample and Chromeleon v7.2 software (Thermo Scientific) was used to quantify peak area. Fatty acids were expressed as percentage by weight of the total fatty acids and represent the means of three technical replicates.

*6.2.4. Near Infrared Spectroscopy*

Spectral data for seeds were collected using a XDS Near Infrared Rapid Content™ Analyzer (Foss, Copenhagen, Denmark) spectrophotometer with an iris adapter insert. Clean seed samples were placed in a crystal ring cup with an outside diameter of 5.1 cm and an inside diameter of 3.8 cm and positioned over the instrument's iris. Approximately 3 g of whole seed were placed in the crystal ring cup and closed with a disposable cardboard back. The wavelength range used was 400-2498 nm with 2 nm resolution used to build calibrations. Using the software WIN ISI version 4.10.0.15326, the results from wet chemistry, GC analysis, and collected NIRS spectral data were analyzed and calibration equations were developed for determining crude

protein, fatty acids, nitrogen, and total oil concentration. For each quality parameter, eight pre-spectra processing methods were used for scatter correction and the same methods were used as mathematical models with modified partial least squares regression with four groups of cross validation (Table 37). Equations were compared by cross validation values (1-VR) and standard error of cross validation (SECV). Evaluation of the equations was conducted using the coefficient of determination ($r^2$).

*6.2.5. Statistical Analysis*

An analysis of variance (ANOVA) was conducted with SAS 9.4 software with the general linear model (GLM) procedure for both wet chemistry and NIRS data to determine if differences between winter- and summer-biotypes were significant (SAS Institute, 2014). Biotype was considered a fixed effect. *F*-protected least significant differences (LSD) at 0.05 significance was used to determine differences between means. Linear regression between predicted and reference values were analyzed and plotted.

6.3. Results and Discussion

The analysis of variance indicated differences ($P \leq 0.05$) between biotypes for crude protein and N concentration when measured with lab analytics. Differences between biotypes were significant for total oil, crude protein concentration and the fatty acid eicosadienoic acid (20:2) when analyzed with NIRS. The rest of the characteristics did not have significant interaction between biotypes (Table 38, Table 39).

Table 38. Analysis of variance and mean squares values for seed crude protein (CP) and total oil concentration, and major fatty acids (16:0, 18:1, 18:2, 18:3, 20:1) obtained by wet chemistry (Lab) and NIRS based on inclusion of 85 accession compared with 173 accessions of camelina seed.

| SOV | df | Lab CP | NIRS CP | Lab N | NIRS N | Lab oil | NIRS oil | df | Lab 16:0 | NIRS 16:0 | Lab 18:1 | NIRS 18:1 | Lab 18:2 | NIRS 18:2 | Lab 18:3 | NIRS 18:3 | Lab 20:1 | NIRS 20:1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Biotype | 1 | 383 | 379 | 98 | 7 | 800 | 1014 | 1 | 0.10 | 0.63 | 0.11 | 1.30 | 3.45 | 3.78 | 0.25 | 2.81 | 1.50 | 0.02 |
| Error | 84 | 72 | 71 | 19 | 22 | 229 | 221 | 171 | 0.23 | 0.15 | 4.59 | 3.38 | 2.89 | 2.34 | 9.02 | 7.73 | 1.34 | 0.79 |
| Significance | | * | * | * | NS | NS | * | | NS | * | NS | NS | NS | NS | NS | NS | NS | NS |
| CV, % | | 8 | 9 | 8 | 9 | 14 | 14 | | 7.55 | 6.17 | 15.35 | 13.18 | 9.21 | 8.28 | 9.10 | 8.4 | 7.72 | 5.96 |

* Significant at 0.05 probability.

Table 39. Analysis of variance and mean squares values for s fatty acids (18:0, 20:0, 20:2, 22:0, 22:1, 22:6, 24:1) content obtained by wet chemistry (Lab) and NIRS based on inclusion of 85 accessions compared with 173 accessions of camelina seed.

| SOV | df | Lab 18:0 | NIRS 18:0 | Lab 20:0 | NIRS 20:0 | Lab 20:2 | NIRS 20:2 | df | Lab 22:0 | NIRS 22:0 | df | Lab 22:1 | NIRS 22:1 | df | Lab 22:6 | NIRS 22:6 | df | Lab 24:1 | NIRS 24:1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Biotype | 1 | 0.001 | 0.083 | 0.01 | 0.05 | 0.03 | 0.16 | 1 | 0.019 | 0.015 | 1 | 0.016 | 0.194 | 1 | 3E-06 | 0.0072 | 1 | 0.033 | 0.0025 |
| Error | 171 | 0.104 | 0.062 | 0.07 | 0.04 | 0.05 | 0.03 | 116 | 0.088 | 0.11 | 171 | 0.31 | 0.18 | 169 | 0.01 | 0.0041 | 47 | 0.013 | 0.0015 |
| Significance | | NS | NS | NS | NS | NS | * | | NS | NS | | NS | NS | | NS | NS | | NS | NS |
| CV, % | | 12.78 | 9.86 | 14.56 | 10.88 | 11.38 | 8.59 | | 63.74 | 71.9 | | 14.68 | 11.16 | | 12.98 | 8.11 | | 28.6 | 9.56 |

* Significant at 0.05 probability.

*6.3.1. Crude Protein and Nitrogen Concentration of Seeds*

Crude protein ranged between 251 and 387 g kg$^{-1}$ (Table 40) across the 85 accessions, based on wet chemistry data. The calibration model developed for crude protein based on this data had a $r^2$ of 0.98, a SEC value of 0.99 and RPD of 9.2 (Table 37, Fig. 9). The high RPD value is indicative of the high prediction ability for crude protein with NIRS. Consequently, it should not be surprising that NIRS values obtained for mean (300 and 316 g kg$^{-1}$) and median (302 and 314 g kg$^{-1}$) crude protein concentration for summer- and winter-biotypes, respectively, were nearly identical to those obtained by wet chemistry (Table 38, Fig. 10).

Table 40. Wet chemistry values and calibration statistics for intact camelina seed quality components.

| Constituent | $n_1$[b] | Range | Mean | SD[c] | $n_2$ | $r^{2a}$ | SEC[c] | RPD[c] |
|---|---|---|---|---|---|---|---|---|
| | | | Wet chemistry values | | | | | |
| Crude protein (g kg$^{-1}$) | 85 | 251-387 | 304.2 | 27.5 | 74 | 0.98 | 0.99 | 9.2 |
| Total oil (g kg$^{-1}$) | 85 | 192-424 | 345.3 | 48.6 | 50 | 0.89 | 0.84 | 4.3 |
| Nitrogen (g kg$^{-1}$) | 85 | 40-62 | 48.6 | 4.41 | 74 | 0.98 | 0.99 | 9.2 |
| Fatty acids (% of oil) | | | | | | | | |
| Palmitic acid (16:0) | 173 | 5.1-8.4 | 6.28 | 0.47 | 108 | 0.47 | 0.30 | 1.7 |
| Stearic acid (18:0) | 173 | 1.8-3.8 | 2.52 | 0.25 | 129 | 0.57 | 0.21 | 1.5 |
| Oleic acid (18:1) | 173 | 9.1-22.1 | 13.92 | 2.06 | 96 | 0.72 | 1.06 | 2.1 |
| Linoleic acid (18:2) | 173 | 15.2-27.1 | 18.46 | 1.69 | 130 | 0.79 | 0.91 | 1.9 |
| Linolenic acid (18:3) | 173 | 22.8-38.4 | 33.06 | 2.90 | 114 | 0.83 | 1.26 | 2.4 |
| Arachidic acid (20:0) | 173 | 1.1-2.7 | 1.91 | 0.28 | 96 | 0.37 | 0.20 | 1.4 |
| Eicosenoic acid (20:1) | 173 | 11.6-18.2 | 14.99 | 1.16 | 114 | 0.59 | 0.68 | 1.6 |
| Eicosadienoic acid (20:2) | 173 | 1.3-2.3 | 1.88 | 0.21 | 117 | 0.41 | 0.14 | 1.5 |
| Arachidonic acid (20:4) | 28 | 0.5-1.6 | 1.34 | 0.22 | 16 | 0.20 | 0.10 | 1.3 |
| Behenic acid (22:0) | 117 | 0.3-3.6 | 0.47 | 0.30 | 85 | 0.20 | 0.03 | 1.3 |
| Erucic acid (22:1) | 173 | 0.8-5.8 | 3.8 | 0.56 | 68 | 0.11 | 0.48 | 1.4 |
| Docosahexaenoic acid (22:6) | 170 | 0.3-0.9 | 0.79 | 0.06 | 118 | 0.35 | 0.22 | 1.1 |
| Nervonic acid (24:1) | 49 | 0.3-0.9 | 0.41 | 0.11 | 34 | 0.09 | 0.49 | 1.4 |

[a]$r^2$ values greater than 0.28 and 0.20 are significant at $P \leq 0.01$ with 83 and 171 degrees of freedom ($n_1$-2), respectively.
[b]$n_1$= 85 samples were analyzed for CP, fat, N, and 173 for fatty acids $n_2$= number of reference values (wet chemistry) used to develop the calibration.
[c]Standard deviation (SD), standard error of calibration (SEC), ratio of performance to deviation (RPD).

Table 41. Near infrared spectroscopy values for crude protein and oil concentration estimates with different number of samples (Summer biotype: $n_1$=63, $n_2$= 149; Winter biotype: $n_1$= 22, $n_2$= 24).

| Constituent | $n_1$ | | | $n_2$ | | |
| --- | --- | --- | --- | --- | --- | --- |
| | Range | Mean | SD | Range | Mean | SD |
| | Summer biotype | | | | | |
| Crude protein (g kg$^{-1}$) | 263-358 | 300 | 17 | 253-358 | 291 | 17 |
| Oil (g kg$^{-1}$) | 278-410 | 351 | 32 | 79-410 | 260 | 85 |
| | Winter biotype | | | | | |
| Crude protein (g kg$^{-1}$) | 249-384 | 316 | 42 | 249-384 | 314 | 43 |
| Oil (g kg$^{-1}$) | 178-427 | 327 | 72 | 178-427 | 323 | 75 |

$n_1$ samples are those matching samples with wet chemistry analysis, and $n_2$ samples are NIRS values estimated with the calibration. There was not enough seed from these samples to do wet chemistry.
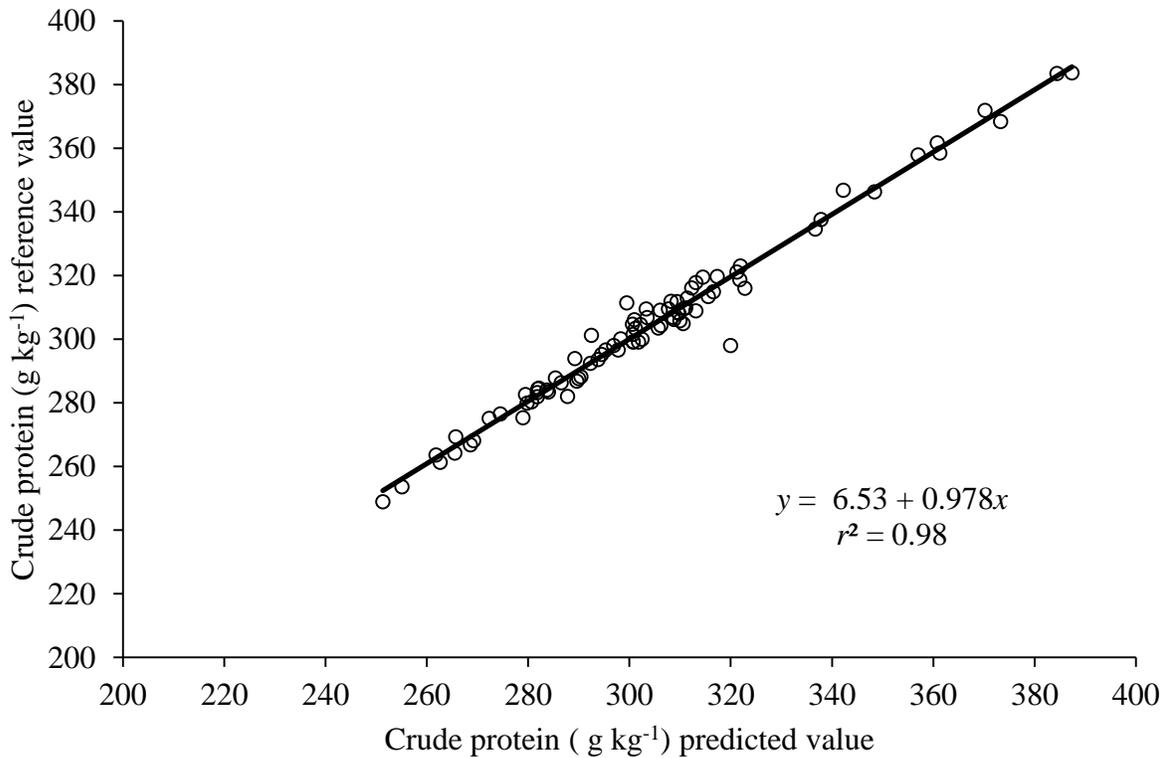


Fig. 9. Crude protein reference vs. near-infrared spectroscopy (NIRS) predicted values ($n$=85).

$$y = 6.53 + 0.978x$$
$$r^2 = 0.98$$

These results indicate that summer-biotypes generally have significantly less ($P \leq 0.05$)

crude protein than winter-biotypes. For nitrogen content, wet chemistry data indicated mean

values of 48 and 50 g kg$^{-1}$, respectively in seeds of summer- and winter-biotypes (Table 42).

Overall, the range of crude protein values observed in both summer- and winter-biotypes are

similar to those reported before by several authors (Zubr, 1997, 2003; Berti et al., 2016; Gesch et

al., 2018).  Previously developed NIRS calibrations for Brassicaceae family oilseeds have

included only two camelina accessions, one summer and one winter out of 367 samples (Oblath

et al., 2016). With the limited number of camelina accessions used in the development of the

calibration of Oblath et al. (2016), it is difficult to know if their predicted oilseed quality traits

for camelina are as comprehensive as the calibration developed in this study.

Table 42. Wet chemistry values for N concentration in summer- and winter-biotypes of camelina
seed (Summer biotype: *n*=149; Winter biotype: *n*=24).

| Constituent | Range | Mean | SD |
|---|---|---|---|
| | | Summer biotype | |
| N (g kg$^{-1}$) | 42-58 | 48 | 3 |
| | | Winter biotype | |
| N (g kg$^{-1}$) | 40-62 | 50 | 7 |

Using the described NIRS calibration equations developed in this study, the remaining 88

accessions of camelina seed (86 summer- and 2 winter-biotypes) not evaluated by wet chemistry

were evaluated for crude protein concentration by NIRS (Table 41). The inclusion of data for the

additional 86 accessions of summer biotypes (149 accessions total) changed the predicted mean

crude protein concentration of summer biotypes from 300 to 291 g kg$^{-1}$ (Table 41), which was

reflected by the 10 g kg$^{-1}$ change in the lower end of the range (Fig. 10). For the winter-biotypes,

the inclusion of two additional accessions had little impact on the mean crude protein

concentration (316 vs. 314 g kg$^{-1}$) and no change in the range of 249 to 384 g kg$^{-1}$ (Table 38, Fig.

10).

*6.3.2. Total Oil Concentration in Seeds*

Total oil content ranged between 192 and 424 g kg$^{-1}$ (Table 40) across the 85 accessions

based on wet chemistry data, with mean concentration greater ($P \leq 0.05$) in seeds of summer-

(351 g kg$^{-1}$) than that of winter- (329 g kg$^{-1}$) biotypes (Table 43). The calibration model

developed for total oil based on this data had an $r^2$ of 0.89, SEC of 0.84 and RPD of 4.3 (Table

38, Fig. 11).

Table 43. Wet chemistry values for oil concentration in summer- and winter-biotypes of
camelina seed (Summer biotype: *n*=149; Winter biotype: *n*=24).

| Constituent | Range | Mean | SD |
|---|---|---|---|
| | | Summer biotype | |
| Oil (g kg$^{-1}$) | 275-424 | 351 | 36 |
| | | Winter biotype | |
| Oil (g kg$^{-1}$) | 192-419 | 329 | 73 |
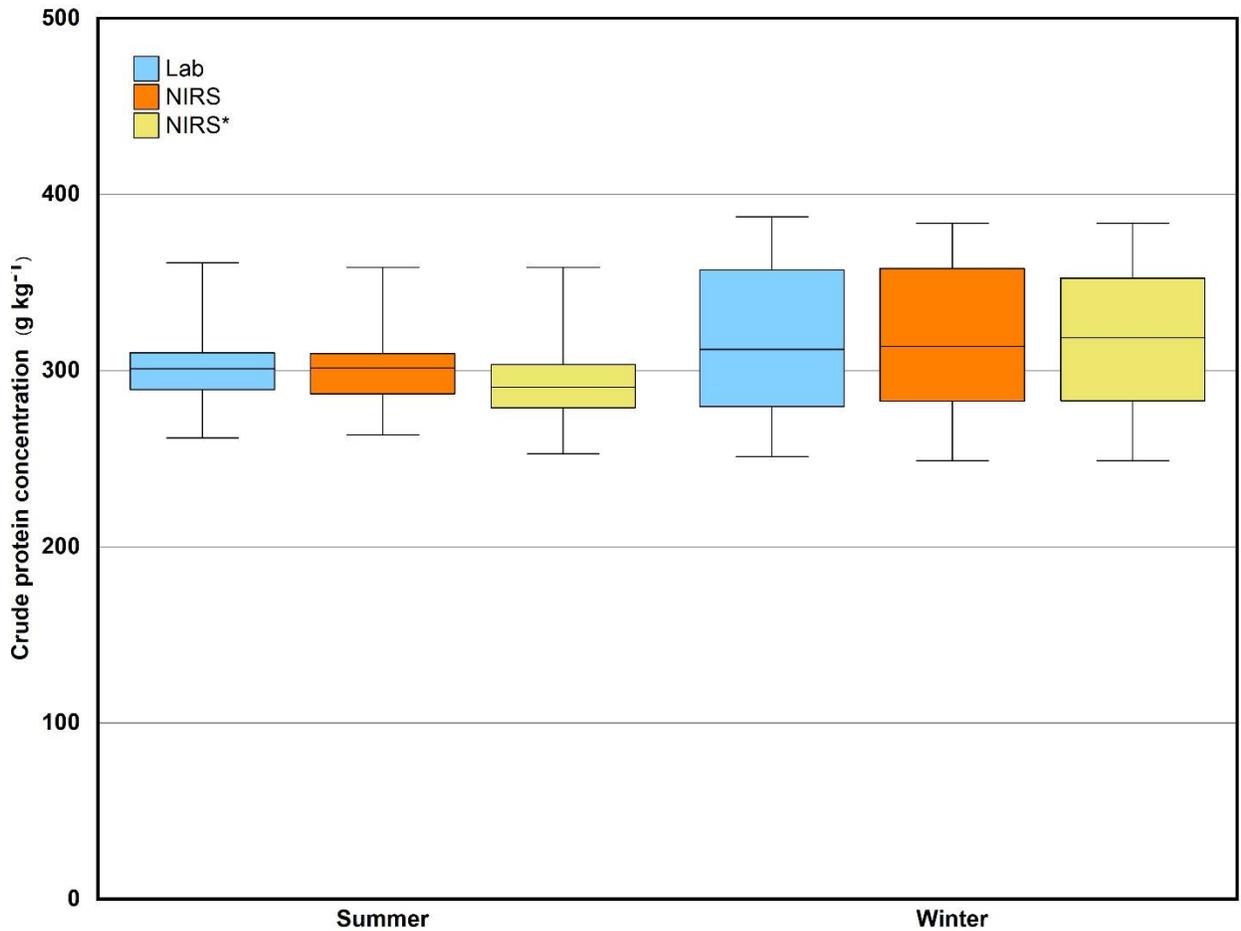
Fig. 10. Crude protein concentration obtained by wet chemistry (Lab) or NIRS from 63 accessions of summer- and 22 accessions winter-biotypes of camelina seed, or NIRS* predicted crude protein concentration obtained from 149 accessions of summer- and 24 accessions of winter-biotypes. Graphs present the interquartile range (boxes) with the line as the median and whiskers as the minimum and maximum values. *(Figure developed by J.V. Anderson).*
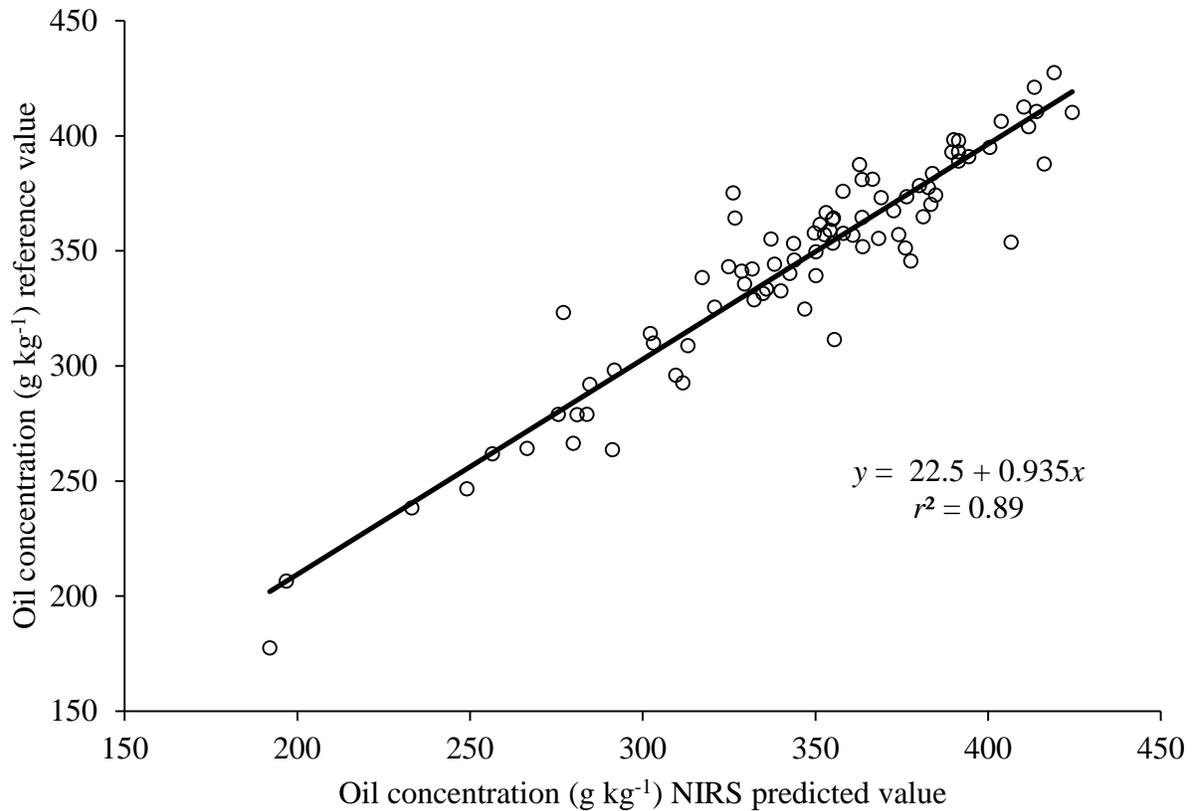
Fig. 11. Oil concentration reference vs. near-infrared spectroscopy (NIRS) predicted values of camelina seeds, *n*=85.

Total oil concentration values of summer- and winter-biotypes obtained with NIRS had a mean (352 and 327 g kg$^{-1}$) and median (355 and 351 g kg$^{-1}$) respectively and were similar to those obtained by wet chemistry (Fig. 12, Table 37). Although the overall mean and median total oil concentration was less in winter-biotypes, total oil concentration in the winter cultivars/lines Joelle, Bison, BSX-WG1, and BSX-WG4 were equal to or greater than the mean value of summer-biotypes and were similar to those previously reported by Gesch et al. (2018). Indeed, based on the 85 camelina accessions used to determine wet chemistry and NIRS calibration equations, the overall variability in the range of total oil concentration was greater among the 22 winter-biotypes compared with the 63 summer-biotypes (Fig. 12).

However, the inclusion of NIRS data for the additional 86 accessions of summer-biotypes with no wet chemistry data, significantly lowered the overall predicted mean total oil concentration of summer-biotypes from 351 to 260 g kg$^{-1}$ (Table 41). The inclusion of two additional winter-biotypes had little effect on NIRS predicted mean total oil concentration.

At this point, it is not clear if these differences in predicted mean oil concentration result from genetic variability of summer- and winter-biotypes, are the environmental influence in which they were grown, time they were harvested, seed age, or a combination of these four factors (Walia et al., 2018). Previous studies (Berti et al., 2011; Gesch et al., 2018) have indicated that the environment does influence seed quality traits such as crude protein and total oil content of camelina. In environments with higher mean temperatures during seed development, oil concentration of camelina decreased (Berti et al., 2011), which has also been observed in other oilseeds. For example, in *Cuphea viscossissima* × *Cuphea lanceolata*, reduction in seed oil concentration was inversely associated with average daily temperature during seed development (Berti and Johnson, 2008). Within the collection of camelina accessions used in this study, accessions having the same pedigree but originating from different geographic locations do appear to have variability for these agronomic traits. Thus, although beyond the scope of this study, further studies should help determine the impact of genotype by environment interaction on these important seed quality traits in summer- and winter-biotypes of camelina.
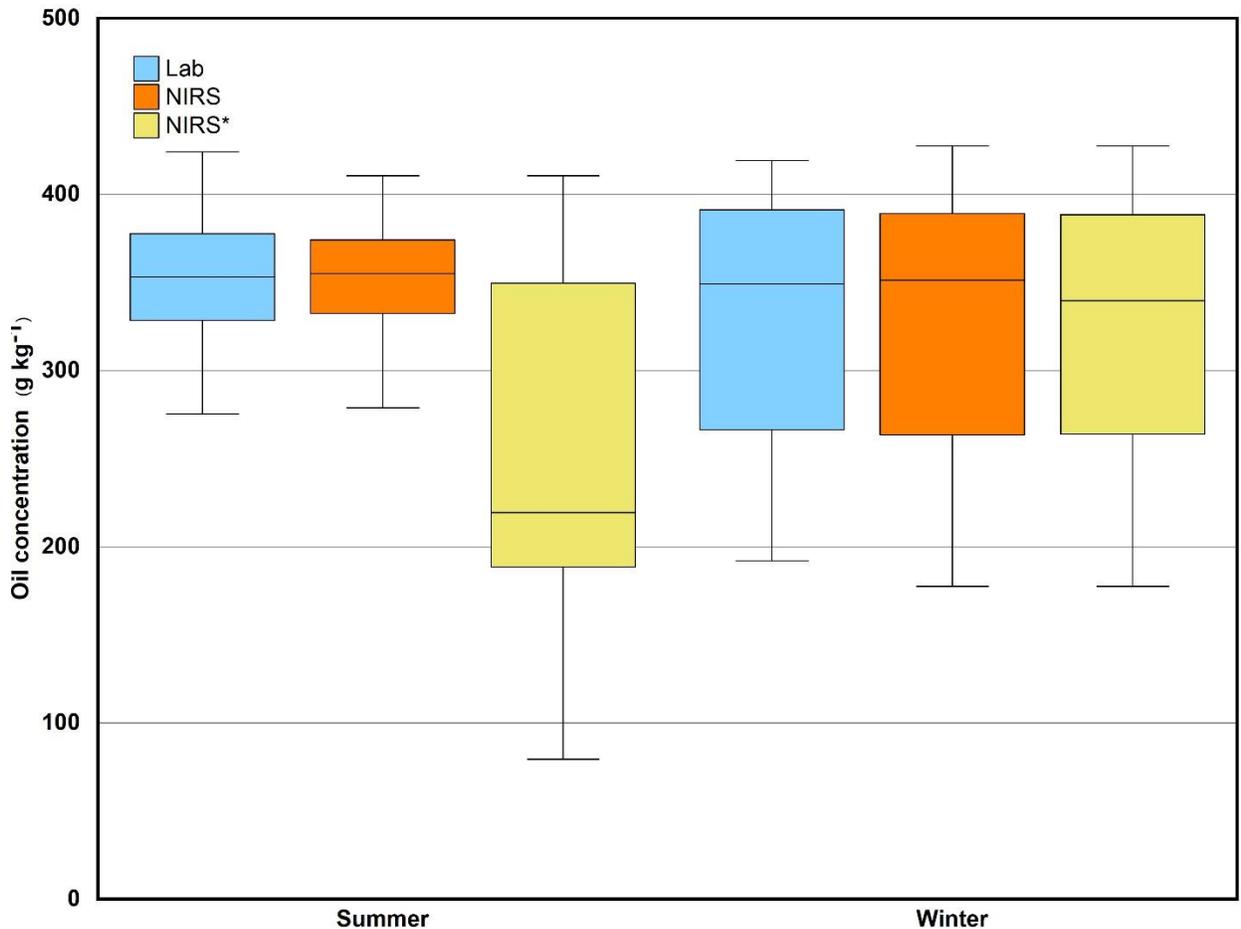
Fig. 12. Total oil concentration obtained by wet chemistry (Lab) or near-infrared spectroscopy (NIRS) from 63 accessions of summer- and 22 accessions of winter-biotypes of camelina seed, or NIRS* predicted total oil concentration obtained from 149 accessions of summer- and 24 accessions of winter-biotypes. Graphs present the interquartile range (boxes) with the line as the median and whiskers as the minimum and maximum values. *(Figure developed by J.V. Anderson).*

### 6.3.3. Fatty Acid Profile

The profile of fatty acids among the 173 accessions tested were similar between summer- and winter-biotypes. However, there was greater variability in the profile of fatty acids from summer-biotypes compared with winter biotypes (Fig. 13), which may be a consequence of the smaller number of winter biotypes evaluated in this study compared with summer biotypes.

The most abundant fatty acid was linolenic acid (18:3) ranging from 22.8 to 38.4%, followed by linoleic acid (18:2) at 15.2 to 27.1%, eicosenoic acid (20:1) at 11.6 to 18.2%, and

oleic acid (18:1) at 9.1 to 22.1% (Table 40). Interestingly, NIRS analysis appear to show less overall variability in individual fatty acid content than that obtained by GC. Calibration models for the main fatty acids; oleic, linoleic, linolenic, and eicosenoic acids had $r^2$ values of 0.72, 0.79, 0.83, and 0.59, respectively, and they all were significant at $P \leq 0.01$ ($df = n$-2) (Fig. 14).

6.4. Conclusion

Near infrared spectroscopy calibrations were developed for crude protein, total oil, and seed oil fatty acid profiles for both winter- and summer-biotypes of camelina. Calibrations for crude protein and oil had excellent performance with $r^2$ values of 0.98 and 0.89, respectively. Based on NIRS predicted values obtained using all 173 accessions, winter-biotypes of camelina appear to have greater overall mean and median oil and protein concentration than summer-biotypes, showing great potential for winter camelina in North Dakota. However, fatty acid profiles were similar between both biotypes. Calibrations for all major fatty acids including linolenic, linoleic, eicosenoic, and oleic acids had good performance with $r^2$ values between 0.59 and 0.83. The calibrations obtained by NIRS in this study will be useful as a non-destructive, high throughput protocol for future determination of seed quality traits in summer- and winter-biotypes of camelina as well as for determining the impact of genotype by environment on these traits. Having non-destructive, high throughput phenotyping protocols for identifying agronomically important traits is also a critical component for conducting genome wide association studies (GWAS), which provides a powerful approach for identifying genetic loci associated with traits of interest.

Fig. 13. Fatty acid profiles (% of total oil) obtained by gas chromatography (GC) analysis or near-infrared spectroscopy (NIRS) from 149 accessions of summer (A)- and 24 accessions of winter (B)-biotypes of camelina seed. Graphs present the interquartile range (boxes) with the line as the median and whiskers as the minimum and maximum values. *(Figure developed by J.V. Anderson)*

Fig. 14. Camelina main seed oil fatty acids reference values vs. near-infrared spectroscopy (NIRS) predicted values ($n$=173).

# CHAPTER 7. CONCLUSIONS

7.1. Winter Camelina Sowing Date

While demonstrating a wide range of acceptable sowing dates, sowing winter camelina in the beginning to middle of September and even as late as early October can have similar seed yield. Fall biomass was not significantly different between sowing dates but ranged from 0 to 733 kg ha$^{-1}$, with more biomass being present at earlier sowi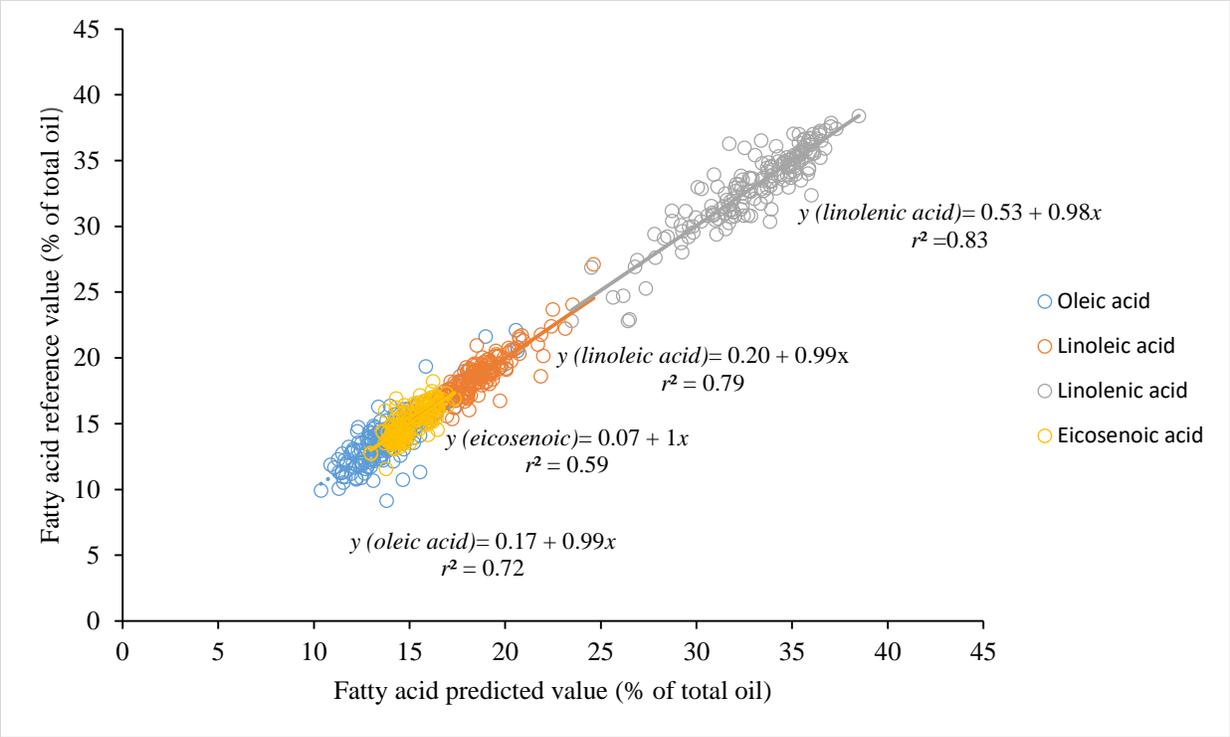ng dates. Winter survival ranged from no plants surviving the winter to 67% of plants surviving. Environmental variables such as suitability of winter and cumulative precipitation can have a significant effect on plant stand establishment and persistence to the next spring. Camelina can effectively remove residual soil nitrate at 15-60-cm prior to winter and again the following spring when sown prior to mid-September. Days to 50% flowering tended to increase with latter sowing dates. Depending on the objective, the sowing date of winter camelina is a critical factor, with sowing prior to mid-September critical for biomass production and reducing soil residual nitrate, while sowing even as late as early October for harvestable seed yield in the following growing season.

7.2. Seedling Morphology and Development

Winter- and summer-biotypes of camelina evaluated in this study had some distinct morphological phenotypes. Significant differences were observed for pairs of vegetative leaves, growth stage, height, total length, width, and number of lobes of the upper most developed leaf, and width of the second most upper leaf between winter- and summer biotypes. These morphological differences can allow both researchers conducting experiments under controlled conditions to determine the correct biotype of unknown seed or producers sowing a questionable seed lot when conditions are favorable for adequate growth in either the fall or spring.

7.3. Seed Characterization and Reflectance Properties

The calibration equation obtained with NIRS for identifying unknown or mixed seed lots of camelina seed proved to have good performance for field grown released cultivar seed lot samples, with ratio of winter seed, but not for seed lot samples grown under controlled conditions. The developed NIRS equation using 119 scanned samples produced an $r^2$ value of 0.96. Furth validation of equations shows that we can differentiate field grown samples at a 95% confidence level. The calibration is extremely useful for determining if a seed lot is a winter- or summer-biotype before seed is sent to producers or planted in the fall and at a fraction of the time and expense of growing out the seeds to determine the biotype.

7.4. NIRS Calibration of Determination Seed Constituents

Near infrared spectroscopy calibrations were developed for crude protein, total oil, and seed oil fatty acid profiles for both winter- and summer-biotypes of camelina. Calibrations for crude protein and oil had excellent performance with $r^2$ values of 0.98 and 0.89, respectively. Based on NIRS predicted values obtained using all 173 accessions, winter-biotypes of camelina appear to have greater overall mean and median oil and protein concentration than summer-biotypes, showing great potential for winter camelina in North Dakota. However, fatty acid profiles were similar between both biotypes. Calibrations for all major fatty acids including linolenic, linoleic, eicosenoic, and oleic acids had good performance with $r^2$ values between 0.59 and 0.83. The calibrations obtained by NIRS in this study will be useful as a non-destructive, high throughput protocol for future determination of seed quality traits in summer- and winter-biotypes of camelina as well as for determining the impact of genotype by environment on these traits. Having non-destructive, high throughput phenotyping protocols for identifying agronomically important traits is also a critical component for conducting genome wide

association studies (GWAS), which provides a powerful approach for identifying genetic loci

associated with traits of interest.

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