# New insights of winter canola survival, seed quality, and yield for the Great Plains region and the United States

by

Mario Ariel Secchi

B.S., National University of Rosario, 2016

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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# Abstract

Canola (*Brassica napus* L.), also known as rapeseed, is an emerging valuable crop with edible oil quality. Climate models forecast erratic temperature and rainfall patterns with contrasting impacts on canola production. The main projected annual changes are linked to increased frequency of extreme heat and drought events during the summer months, challenging the overall production of row crops. The adoption of winter oilseed crops such as winter canola could be a feasible option to overcome these challenges and to diversify agricultural systems.

This dissertation is organized in four chapters which explore in detail the main challenges and potential expansion of winter canola production. Chapter 1 provides an overall introduction and context in production for this crop. Chapters 2 and 3 summarize a series of multi-environment studies with more than 25 states and 200 genotypes (during the 2003-2018 period) providing critical information for breeding programs, agronomy management, and the future direction of canola production. Chapter 4 compiles information from a total of 37 papers gathering 1794 observations to execute a meta-analysis on the effect of heat and drought on the formation of seed yield and quality on canola crop. Chapter 5 presents the impact of future weather changes (focused on temperature and precipitation) on seed yield via the utilization of crop growth models to provide an assessment on potential yield shifts across the US.

In summary, this dissertation provides critical information identifying potential environments suitable for winter canola production. New insights ranging from improving our understanding of winter canola survival, geographical variation for yield and oil productivity, impacts of critical stressors such as heat and drought on seed yield and quality traits, and lastly, future weather impact on seed yield across the US is assessed to evaluate geographical changes in production and to develop potential mitigation strategies.

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Approved by:

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# Abstract

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# Dedication

*To Mónica, Elsa, Verónica, Melisa, Tita, and Mario Alberto...*

# Chapter 1 - Introduction

As a result of the green revolution, a loss of genetic diversity of crops has been reported (Jacques & Jacques, 2012). Climate change is expected to compromise future food demands in our current agricultural systems, and without any changes, up to a third of the world population could face hunger by 2050 (Hasegawa et al., 2021). In North America, maize (*Zea mays* L.) - soybean [*Glycine max* (L.) Merr.] rotation is one of the most common cropping sequences (Vanhie et al., 2015). Recent studies highlight the importance of increasing crop diversity in current agricultural systems to meet sustainability goals (Renard & Tilman, 2019). Although overall climate change trends are expected to decrease crops yields, recent studies reported a large variation across geographical regions and crop species (Aggarwal et al., 2019; Challinor et al., 2014; Hasegawa et al., 2022). In this context, research efforts should focus on understanding how current agricultural systems could overcome future climate change risks. Identifying the environmental drivers for food production and selection of crop species will assist in increasing crop diversification and could mitigate climate change's impacts on food security.

Among several oilseed crops, soybeans are the largest global production, while rapeseed rank second (FAOSTAT, 2022). Canola (*Brassica napus* L.) is a regulated rapeseed crop with edible oil quality standards and rising demand, mainly used as a source for vegetable oil, high protein animal feed, and biodiesel. The largest global producer of canola during the 2018-2020 period was Canada, with canola planted in the spring and harvested in summer (spring types) (FAOSTAT, 2022). One of the major worldwide canola consumers is the United States (US), although, the US only produced 5.2% of global production in 2019, also mostly with spring types in the northern Great Plains region of North America (FAO, 2020; USDA-NASS, 2019).

From a food security standpoint, the study of projections on climate change is relevant to assessing the potential impacts in our food systems. Projections for climate change forecast an increase on precipitations during winter and spring months, and a higher frequency of extreme drought and heat events during summer for most of North America (Almazroui et al., 2021; Qian et al., 2018). Therefore, the adoption of winter crops could be a feasible option to mitigate the negative impacts of climate change on crop production. Winter canola (bi-annual winter type sown in the fall and harvested in summer) has the potential of adjusting to these future weather conditions, helping to satisfy the increasing demand of edible oil and biofuel feedstock. Inclusion of winter canola, as an alternative to winter wheat (*Triticum aestivum* L.), has been proven to break weed and pest cycles (Angus et al., 2015; Kirkegaard et al., 2016). Therefore, it is critical to study yield-limiting factors, response to abiotic stress conditions, and future weather impacts with the goal of studying the potential expansion of winter canola within the US states.

Winter canola hectares in the Southern Great Plains declined in the last few years (USDA-NASS, 2021) due to loss of productivity, consistency of yield and oil concentration, and other farming challenges, such as fall stand establishment and winter survival (Assefa et al., 2014) limiting winter canola production. The environment plays a key role influencing survival, but in-depth evaluation of meteorological factors has not yet been conducted. Canola seed yield and quality (oil, protein, and fatty acids profile) can be affected by temperature and water stresses (Si et al., 2003, Faraji et al., 2009; Farré et al., 2001). Contrasting abiotic stresses impacts (negative, neutral or positive) on canola seed yield and quality are reported in the literature (Pokharel et al., 2021; Aslam et al., 2009, Zarei et al., 2010). Refining our understanding of the environmental drivers of canola seed yield and quality; and identify optimal genotype, environment, and management (G x E x M) combinations, will assist in providing new opportunities to expand canola production, increase crop diversification, and satisfy edible oil and biofuel demands.

Therefore, the general objectives of the research present on the following chapters of this dissertation are to: identify meteorological factors defining winter canola survival to build probabilistic models and define areas of current germplasm adaptation with low winterkill (Chapter 2); explore the main drivers of oil concentration variability and identify US regions suited to winter canola production with high final oil yield (Chapter 3); quantify heat and drought impacts on canola seed yield and quality (oil and protein) (Chapter 4); and quantify the impacts of future climate scenarios on winter canola seed yield over time and identify US regions suitable for future expansion of winter canola production (Chapter 5).

In Chapter 1 of this dissertation, the main research context and significance has been introduced, while the objectives have been identified. In Chapter 2, the main drivers of winter survival will be reviewed to build models that will identify regions with quantified probabilities of achieving different levels of winter survival. Then, current germplasm adaptation and potential future varieties development will be defined. In Chapter 3, the US environmental variability of winter canola seed oil concentration will be characterized, discussing the development of varieties with both high seed yield and oil concentration. In Chapter 4, the magnitude and direction changes on seed yield and quality driven by abiotic stresses will be quantified. The impact of heat and drought timing and duration on canola seed yield, yield components, quality, and oil yield will be discussed. In Chapter 5, simulations using crop growth models will evaluate the impact of future climate scenarios on yields. Lastly, Chapter 6 presents a summary with the main findings and limitations encountered on the research from this dissertation, and on what future research projects should focus will be presented.

# Chapter 2 - Winter survival response of canola to meteorological variables and adaptative areas for current canola germplasm in the United States

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## Abstract

The introduction of winter canola (*Brassica napus* L.) into rotations with winter wheat (*Triticum aestivum* L.) in the United States (US) revealed economic and agronomic benefits as well as improved weed and pest control. Canola stand establishment during the fall and plant survival over the winter are critical to the success of this crop. The environment plays a key role influencing survival, but in-depth evaluation of meteorological factors has not been conducted. This research study aimed to i) identify meteorological factors underpinning winter canola survival, ii) build probabilistic response models based on historical meteorological data for different severities of winter kill across the US, and iii) define areas of adaptation of current germplasm. A winter survival dataset was compiled from the National Winter Canola Variety Trials from 2003 until 2018 (190 site-years) and auxiliary meteorological data over the last 40 years. A regression tree analysis indicated that meteorological variables related to minimum temperature, fluctuating temperatures above and below 0° C, and windchill during the cold period were the main factors accounting for winter kill. Cold periods across all site-years were classified into three clusters: cold periods with high (96%), medium (70%), and low (28%) average plant winter survival. For 94 US sites, the probabilities of these conditions were calculated and summarized in a map that defined areas of adaptation: a large area south of 35° N latitude for the US was identified with greater potential for overwintering success. Based on the

response under multiple meteorological conditions, four distinct genotype survival groups were identified (tolerant, semi-tolerant, semi-susceptible, and susceptible). Groups with a greater number of genotypes differ in the impact of meteorological conditions on survival for the medium cluster. In regions with more favorable conditions for overwintering success, farmers may be open to introducing this crop to diversify their farming system.

## 2.1 Introduction

The United States produced 3.6 million tons of canola (*Brassica napus* L.) in 2019, representing 5.2% of global production (FAO, 2020). Although the state of North Dakota harvested 83% of US canola as spring canola (USDA-NASS, 2019), other southern US states have the potential to introduce winter canola, also known as winter oilseed rape or double-low rapeseed, where winter wheat (*Triticum aestivum* L.) is the only option as a winter crop or as a service crop to diversify the current maize (*Zea mays* L.)-soybean (*Glycine max* [L.] Merr) farming system. Since the 1970s, a loss of genetic diversity of crops has been reported as a result of the Green Revolution (Jacques & Jacques, 2012). Inclusion of winter canola into farming systems with winter wheat, as opposed to mono-cropping, has proven to break weed and pest cycles (Bushong et al., 2012). Increasing crop diversity in current agricultural systems can be the only path to meeting sustainability goals (Renard & Tilman, 2019).

Fall stand establishment and winter survival have been suggested as key limiting factors to the success of winter canola production (Assefa et al., 2014). Broadleaf winter survival is a complex trait, and several stresses influence this process such as prolonged exposure to subzero temperatures, ice encasement, diseases, wind desiccation, and soil heaving (Levitt, 1956). Plant tolerance to environmental stresses decreases as resources necessary for plant survival become depleted throughout the winter (Gusta et al., 1982). Temperature and precipitation during the winter period are critical for effective winter survival (Fowler et al., 1981; Waalen et al., 2013).

More in-depth studies for this trait in winter canola were carried out in Europe. Salmon, (1918) identified temperature and soil moisture as critical factors for winter kill of grain crops. Rainfall and temperature during the winter season, which can vary dramatically among years and sites, are key environmental variables in current farming systems (Assefa et al., 2014). Although there is no clear understanding of genotype by environment interaction effects for winter survival in the US, producers have concerns regarding low plant establishment and winter survival (George et al., 2012; Stamm & Watson, 2013).

A significant breeding effort has increased the winter hardiness of canola genotypes (Rife et al., 2001; Stamm et al., 2015). Because of the complexity of the trait, a comprehensive understanding of how meteorological factors impact winter survival and yield should be pursued to facilitate winter canola production in new areas. This understanding will facilitate breeding to expand the area of adaptation and production and could be integrated with whole-genome prediction methodology (Messina et al., 2018). While a few site-specific winter survival analyses were conducted (Holman et al., 2011; Waalen et al., 2013), a comprehensive synthesis of meteorological variation in the US and its influence on this critical plant trait is lacking. Building foundational knowledge of crops such as canola and other grain, oil, or service crops will be required to transform current (undiversified) farming systems.

The overall objective of this work is to determine the area of adaptation of current winter canola germplasm based on winter survival. This knowledge will facilitate crop diversification, breeding efforts, and genetic evaluation that may feed the expansion of the area of adaptation. The specific aims of this research study were to i) model winter canola survival based on meteorological factors, ii) build probabilistic response models based on historical meteorological data for different severities of winter kill, and iii) define areas of adaptation of current germplasm across the US. A large dataset compiled from the National Winter Canola Variety

Trials (NWCVT), conducted from 2003 to 2018 across 94 unique sites with auxiliary weather data for each site-year were assembled and analyzed.

## 2.2 Materials and Methods

### Data and predictors

#### *Field trials*

The field dataset was curated from the National Winter Canola Variety Trial (NWCVT) (Stamm et al., 2019). The purpose of this national network of trials is to evaluate canola winter survival, yield performance, and other important agronomic traits on multiple varieties across various US states. The trials also aim to find suitable areas of adaptation of new genotypes and increase the visibility of winter canola across the country. The field dataset comprises 94 sites covering 23 US states, spanning 2003 through 2018, for a total of 333 site-years. Only site-years with information on winter survival were included, decreasing the number of sites and site-years to 54 and 191, respectively. The experimental design for each trial defined by site and year was a randomized complete block with three or four replications. The number of genotypes included in any given site and the number of replications varied depending on the site and year. Genotypes included in each site and year changed based on maturity and year of commercialization. Winter survival ratings were measured after dormancy was completed, or approximately when the average daily temperature exceeded 4 °C. This coincides with the period of rapid, new leaf development, just before the plant enters the reproductive phase at bolting, and when the threat of further losses is low. Winter survival is a visual estimate of the percentage of plants alive in spring relative to those present before the winter period (Stamm et al., 2012). Based on field conditions up to the point of rating winter survival, the plants that do not initiate new leaf growth are observed as lost to winter kill.

### ***Weather dataset***

Daily weather data were extracted from Google Climate Engine (Huntington et al., 2017) for the period 1979-2019 (40 years) for each site. The variable set included daily minimum and maximum temperatures (°C), precipitation (mm), wind velocity (m $s^{-1}$), solar radiation (W $m^{-2}$), vapor pressure deficit (kPa), and evapotranspiration (mm) calculated using alfalfa (*Medicago sativa L.*)  as reference. The daily weather data was utilized to i) create secondary weather variables to characterize winter harshness; ii) identify the beginning and end of the cold period (CP); iii) build a model to predict meteorological survival clusters with meteorological variables during the CP, and iv) predict meteorological survival clusters and calculate the probability of occurrence over 40 years. For each site-year, secondary daily weather variables were created by calculating means and difference between maximum and minimum for temperature (°C) and growing degree days (°C.d.) (Table 2.1). Mean temperature was then utilized to identify the beginning and end of the CP as the period when temperatures were below 0 °C. An example of the mean daily temperatures preceding, during, and after the CP for the Manhattan, KS, 2010-2011 growing season is shown in Figure 2.1.

### ***Predictors***

After the CP was determined for each site-year, the meteorological data was filtered by date to contain only information within this period. Secondary summarized meteorological variables were calculated including CP duration days (N), number of times when mean temperature shifts from negative to positive or vice-versa (ncycle), slope between cumulative GDD (with minimum base temperature of 0 °C and maximum temperature of 30°C) and days after planting (Slope), and class descriptors for mean temperature (Table 2.2). All daily variables were summarized by averaging, summing, extracting minimum and maximum values, or counting within the CP (Table 2.2). Windchill calculations were performed following the equation presented by (Osczevski & Bluestein, 2005).

### Analyses

A series of analyses divided into seven steps were conducted to estimate the contribution of genotype (G), environment (E), and interaction (G × E) to the overall variance for canola winter survival (S). Because S was not normally distributed, we used a log transformation. Once variance components were estimated (step 1), a mixed linear model was utilized to estimate the E and G × E effects (step 2). Predictions from this model, Best Linear Unbiased Estimators (BLUEs) for E effects, and Best Linear Unbiased Predictors (BLUPs) for the G × E effects were produced by solving for model-based E marginal means and G × E variance components, respectively. Environment BLUEs for S were transformed into survival classes using cluster analyses (step 3). These categorized data were used in modeling meteorological survival class as a function of meteorological variables (step 4) using conditional inference. Step 5 used the model developed in step 4 to simulate meteorological survival class as a function of meteorological predictors and classify each site-year combination for the risk level of S. Step 6 repeats G × E analyses using meteorological survival class as predictors rather than site-year as factor. BLUEs from step 6 were transformed into 4 genotype survival classes. A final mixed model was used to model S as a function of E and G survival classes and their interaction (step 7).

In step 1 we modeled the data using a random-effect model (equation 2.1) (n=23,225),

$$\mathbf{log}(\boldsymbol{S}) = \overline{\boldsymbol{G_\iota}} + \overline{\boldsymbol{E_J}} + \overline{\boldsymbol{GE_{\iota J}}} + \boldsymbol{\varepsilon_{ijk}}\ , \qquad \textbf{Equation 2.1}$$

where all terms were considered random effects with error $N\sim(0,\sigma)$ and a general symmetric positive-definite variance-covariance matrix structure. E here is defined by the site-year identifier.

Step 2 used a mixed model (equation 2.2),

$$\mathbf{log}(\boldsymbol{S}) = \overline{\boldsymbol{G_\iota}} + \boldsymbol{E_j} + \overline{\boldsymbol{GE_{\iota J}}} + \ \boldsymbol{\varepsilon_{ijk}}\ , \qquad \textbf{Equation 2.2}$$

where E was considered a fixed effect, and G and G × E considered random effects. G was considered a random effect as the inference is over a sample of genotypes and not any genotype in particular. E here is defined by the site-year identifier.

In step 3, environment BLUEs for survival, on the response scale, (*n*=190) were clustered into three groups: poor, medium, and high; using k means algorithm. The optimal number of clusters (i.e., k=3) was selected by testing k values from 1 to 10 and choosing the one with the most votes from 30 different indices. The categorical meteorological survival class ($MS_c$) was integrated with meteorological predictors as described above.1

In step 4 we used the dataset containing 39 summarized meteorological predictors (Table 2.2), to model meteorological survival class $MS_c$ (n=190) as a function of meteorological factors using conditional inference tree methodology. The modeling process included model parameterization and model fitting. During model parameterization, the model with the best values for the hyperparameters of maximum depth and alpha was found by performing leave-one-out cross-validation. Maximum depth controls the number of horizontal node layers of the tree, and alpha controls the significance level for a variable to be selected to enter the tree. Maximum depth values of 2, 3, 4, and 5 were evaluated along with alpha values of 0.01, 0.02, 0.03, 0.04, 0.05, and 0.1. Hyperparameter values of maximum depth=4 and alpha=0.1 were then chosen based on overall classification accuracy. Thereafter, model fitting was conducted using all the data and the hyperparameters were calculated in model parameterization. Leave-one-out cross-validation was conducted to estimate the model performance metrics of overall and category-specific accuracy, sensitivity, and specificity. Using the classification tree was possible to predict meteorological survival class based on meteorological covariates (M$S_{ce}$).

Step 5 utilized long-term daily weather records (1979-2019 period) for each of the 94 sites included in the multi-environment trials to estimate the area of adaptation of current canola germplasm based on the risk of survival. The long-term meteorological data was processed in the same manner as described under *Predictors*. Only years with a CP (mean temperature below 0$^{o}$ C) were kept, causing the number of years per site to vary between 19 and 40. In this simulation step, the trained tree model was used to predict the CP of each site-year (M$S_c$) into a risk class ($S_r$), poor, medium, and high, based on the simulation for all years for each site. The original data set (n=23,225) was integrated with predictions for M$S_c$. Only genotypes present at least once in each of poor, medium, and high meteorological survival clusters were kept (n=19,919).

In step 6, a mixed-effect model was fitted to the data (equation 2.3),

$$\log(\boldsymbol{S}) = \boldsymbol{G_i} + \boldsymbol{MSc_j} + \boldsymbol{GMSc_{ij}} + \overline{\boldsymbol{E_k}} + \overline{\boldsymbol{Eb_{kl}}} + \boldsymbol{\varepsilon_{ijklm}}\,, \qquad \textbf{Equation 2.3}$$

with G and MSc defined as survival class and their interaction G × M$S_c$ considered a fixed effect, and block ($b$) nested within the site-year random effect. Pairwise comparisons on BLUEs for genotype by M$S_c$ interaction were performed across meteorological survival clusters using alpha=0.05. Based on the pairwise comparison letter separation result, four distinct genotype survival behaviors across meteorological survival clusters were identified, hereafter referred to as genotype survival group G$S_g$.

Step 7 used a mixed-effect model (equation 2.4),

$$\mathbf{log}(\boldsymbol{S}) = \boldsymbol{GSg_i} + \boldsymbol{MSc_j} + \boldsymbol{GSgMSc_{ij}} + \overline{\boldsymbol{E_k}} + \overline{\boldsymbol{Eb_{kl}}} + \overline{\boldsymbol{EG_{km}}} + \boldsymbol{\varepsilon_{ijklmn}}\,, \qquad \textbf{Equation 2.4}$$

where $GSg_i$, $MSc_j$ and their interaction were fixed effects, and block ($b$) and genotype nested within the site-year random effect. Pairwise comparisons for $GSg_i \times MSc_j$ were performed within $MSc_j$ using alpha=0.05.

***Software***

All analyses were conducted within the R framework (R Core Team, 2019). Mixed model analyses were solved using the function lmer from package lme4 (Bates et al., 2015b). Tree models were developed using the function *ctree* (Hothorn et al., 2006) included in the package *partykit* (Hothorn & Zeileis, 2015). Cluster analyses were conducted using the functions kmeans included in the R package stats, with Euclidean distance, and the final number of clusters was determined with the function *NbClust* included in the package *NbClust* (Charrad et al., 2014).

## 2.3 Results

This study sought to understand the contribution of G, E and G × E on the determination of total variation on winter survival of canola. Results from the analyses of variance components showed that G, E and G × E explained 3%, 71%, and 7% of the variation in winter canola survival, respectively. The rest of the variation was pooled into model residuals (19%). The effect of G × E is twice as large as G indicating that G × E is an important determinant of survival. However, the sum of both terms (10%) is just a small proportion of the variation explained by E. Later sections of this paper will focus on modeling the environmental determinants of survival, and to a lesser degree on the model to explain G × E.

Overall, winter survival averaged 84%, but ranged from 0 to 100%, indicating a broad range of variation (Table 2.3). Site-year modeled survival was grouped into poor, medium, and high survival clusters. The minimum, mean, and maximum survival and number of site-years per survival cluster were 0, 28, 48, and 27 for the poor; 50, 70, 82, and 36 for the medium; and 83, 96, 100, and 127 for the high, respectively. Summary statistics for meteorological variables during the CP had large variability, with coefficients of variation ranging from 14% (meanWind) to 564% (Coldest_pct). The wide range in coefficients of variation for all meteorological variables was expected due to the geographical extent of the dataset, ranging from Texas to northern Minnesota and from eastern North Carolina to Washington.

The conditional inference tree classified site-year CPs into seven terminal nodes based on the evaluation of meteorological variables (Figure 2.2). The most relevant variables classifying the CP of 190 site-years into meteorological survival clusters and its specific binary splits of the final tree model were in the order of importance from high to low: i) Colder (5 days), ii) ncycle (24 cycles), iii) cET (465 mm cumulative ET), iv) meanWindchill ( -3.6 °C), v) Colder_pct (3%), and vi) maxTmean (13.4 °C); (see Table 2.2 for full description of the predictors). The leave-one-out cross-validation procedure resulted in a model fit with overall accuracy of 58%. Accuracy for the poor, medium, and high meteorological survival clusters were 48%, 40%, and 60%, respectively. Category-specific sensitivity for the same classes were 10%, 0%, and 71%, and for specificity were 85%, 79%, and 49%, respectively. Site-year CP was classified as poor, medium, and high survival clusters in terminal nodes 12 (n=31); 7 and 13 (n=19), and 4, 6, 8, and 10 (n=140), respectively.

The classification model was used to classify each CP for all 94 sites during the 1979-2019 period, into one of the meteorological survival clusters. Thereafter, the proportion of each meteorological survival cluster overall years (from 19 to 41 years depending on the site), was calculated for each site (Figure 2.3). Overall, sites with a greater proportion of poor, medium, and high meteorological survival were found at latitudes >39° N, between 35° N and 39° N, and <35° N, respectively.

Canola winter survival was determined by meteorological survival cluster alone and as part of the interaction with genotype ($p<0.0001$). Survival means were extracted across meteorological survival cluster as this was the most relevant type of comparison. BLUEs for genotype plus genotype by $S_r$ interaction led to the identification of four different genotype-specific behaviors: tolerant (7 genotypes), semi-tolerant (129 genotypes), semi-susceptible (56 genotypes), and susceptible (10 genotypes) (Figure 2.4).

BLUEs for G plus G × E by meteorological cluster show that all four genotype survival groups had similar mean survival in the high meteorological survival cluster (from 95% to 98%, Figure 2.5). In the medium meteorological survival cluster, survival was greatest in tolerant and semi-tolerant genotype survival groups (78% and 75%, respectively), and lowest in the susceptible group (30%). In the poor meteorological survival cluster, only the tolerant genotype survival group had a significant greater survival (40%), while the other genotype survival groups ranged from 17% to 19% survival.

A breakdown of the genotype survival groups provides insight into the current state of winter canola genotype development and testing in the US. The susceptible canola genotypes are a mix of commercial and experimental genotypes bred outside the US that were grown in the country briefly before more adapted materials could be accessed. The semi-susceptible genotype survival group contains many experimental and commercial genotypes developed outside the US, but also some of the first genotypes developed specifically for US environments. This group contains the first genotypes to be grown on a widespread basis and the very first hybrid to be introduced to the market. The semi-tolerant genotype survival group contains many experimental and commercial genotypes, but the majority are from past and current US breeding programs.

This includes a large number of experimental and new commercial genotypes from the Kansas State University breeding program, which has the goal of improving winter hardiness since its conception in the mid-1990s. Newer hybrids from Europe with enhanced levels of winter hardiness and those containing the semi-dwarfing trait, a trait of significant benefit to winter survival in harsher European environments, are also a large proportion of this group. Other group members include widely grown US open-pollinated genotypes and popular European commercial hybrids. The tolerant survival group contains a few foundational breeding lines from the Kansas State University breeding program and the most winter hardy, commercially available genotypes on the market today. More specific information on the genotypes such as name, type and decade of release is provided in Table A.1.The most evenly distributed groups over all the evaluated states were the semi-tolerant and semi-susceptible genotyped groups (Figure 2.6).

## 2.4 Discussion

Understanding the impact of meteorological factors on survival of winter canola will help to define breeding and agronomic objectives to close yield gaps. At the same time, mapping meteorological risk for winter survival will not only facilitate the introduction of winter canola within current cropping systems but improve overall diversity and sustainability. Although managing a new crop can be a difficult task, winter canola offers an alternative for sites where winter crops are limited to one species. However, some site-specific factors can limit its production including: available agronomic and varietal information, producers willing to grow a new crop, delivery points within a reasonable transportation distance, obtaining a good stand at planting, heat stress at flowering, and challenges at harvest caused by shattering of pods (Stamm & Watson, 2013). Identifying winter canola genotypes that will overwinter and the optimum planting date for a given region are two critical steps that must be resolved before the crop can be introduced into new areas (Holman et al., 2011b). The two larger winter survival groups indicate the broad adaptation of semi-susceptible and semi-tolerant genotypes among geographical regions in the US. This indicates the potential for the development of more tolerant genotypes for new areas. Even though this study was one of the first to provide a comprehensive analysis of winter canola survival, a few factors limited our quantitative evaluation: i) lack of quantification of plants before and after winter, which increased the subjectivity of the evaluation; and ii) quality of weather data. Even though gridded data seemed to be robust for temperature (Mourtzinis et al., 2017), other weather variables may have been less accurately estimated.

The outcomes presented here on winter canola survival provide foundational knowledge for canola breeding processes to select genotypes better adapted to cold environments. These results may give breeders more ways to quantify the "type" of winter kill they observe. (Waalen et al., 2013) provided one of the first in-depth characterizations of meteorological factors affecting winter canola survival in Norway, emphasizing not only the effect of the stress but also the importance of timing. Temperature fluctuations in the US Great Plains during the winter seem to trigger phases of dormancy and re-growth, creating significant stress on the plant (Rife & Zeinali, 2003). Cold acclimation (exposure to low temperature for temperate plants to achieve maximum freezing tolerance), de-acclimation (fully cold-acclimated plants are exposed to warm temperatures), and re-acclimation (re-exposure to cold acclimating temperatures) are complex processes studied in-depth in Canada (Trischuk et al., 2014). The "perfect" sequence of events to reduce winter kill might be to enter into growth cessation with adequate cold acclimation processes, followed by a winter period without extreme events of freezing temperatures, and finishing with a slow and gradual growth elongation and de-acclimation period. This agrees with (Rapacz, 1998), who showed that oilseed rape almost doubled its frost resistance through growth cessation during cold acclimation. In addition, Rapacz (2002) demonstrated in central Europe

(Poland), that further re-acclimation is limited if bolting has begun during de-acclimation. Based on our findings, winter survival was negatively affected by more than 24 cycles of mean daily temperature shifting from negative to positive or vice-versa, followed by cold temperatures with wind chill temperatures below -3.6 °C. According to (Levitt, 1972), elongation growth may interfere with cold acclimation as a result of competition for photo assimilates between growth and acclimation, thus, the plant may be more susceptible to frost due to greater water content in the seedling. (Rife & Zeinali, 2003) reported that de-acclimated seedlings could be re-acclimated, with the accumulation of dehydrins in canola linked to the development of frost tolerance (Schilling, 2004). Likewise, carbohydrate concentration increased during cold acclimation in winter canola (Trischuk et al., 2014) correlating to the photosynthetic capacity of the plants (Hurry et al., 1995).

In summary, plant, meteorology, and management factors such as days without snow cover, root collar diameter, the height of the crown (rosette) at the beginning of the winter, ice encasement, topography, conditions at planting, stand establishment (Trischuk et al., 2014; Waalen et al., 2013), plant density, crop residue on the soil, leaf development (Lääniste et al., 2007), dehydration during sunny and/or windy days while the soil is frozen (Sovero, 1993), prolonged exposure to subzero temperatures, diseases, and soil heaving (Levitt, 1956) may be involved and interact to influence this important plant trait. This evidence, mostly from northern regions such as Europe and Canada, along with our results, suggests that winter survival is a complex trait.

Future research should focus on improving winter survival measurements, integrating new technologies to improve rapid phenotyping with the goal of increasing standardization and precision, and reducing the subjectivity, labor, and time to collect data for this relevant trait of canola. Lastly, investing resources to understand the physiological processes underpinning this trait and its interaction with other factors such as meteorology, management, and genotype will be relevant for increasing productivity and stability of yield over time.

## 2.5 Conclusions

Our analysis of National Winter Canola Variety Trial data indicated that during the winter period, the most relevant meteorological variables affecting winter survival were related to minimum temperature and its fluctuations. There are three important outcomes of this study. First, we found that the number of days with temperatures between -10°C and -15°C, the number of cycles when the temperature fluctuates above or below 0°C, and wind chill temperature during the cold period were the main meteorological variables that explained mean winter survival across 190 site-years. Second, we documented the potential to have near 100% winter survival below 35° N latitude in places where vernalization requirements could be satisfied, as well as in Minnesota, Washington, western Colorado, and near the Pacific coast. Third, we found that most broadly adapted genotypes were classified as semi-tolerant (129) and semi-susceptible (56) to winter kill in the US, indicating there is potential to develop more tolerant genotypes for new areas and widespread use of semi-tolerant genotypes exists. Caution should be taken in specific states between 35° N and 40° N latitude where continental conditions are highly diverse and winter survival can be problematic.

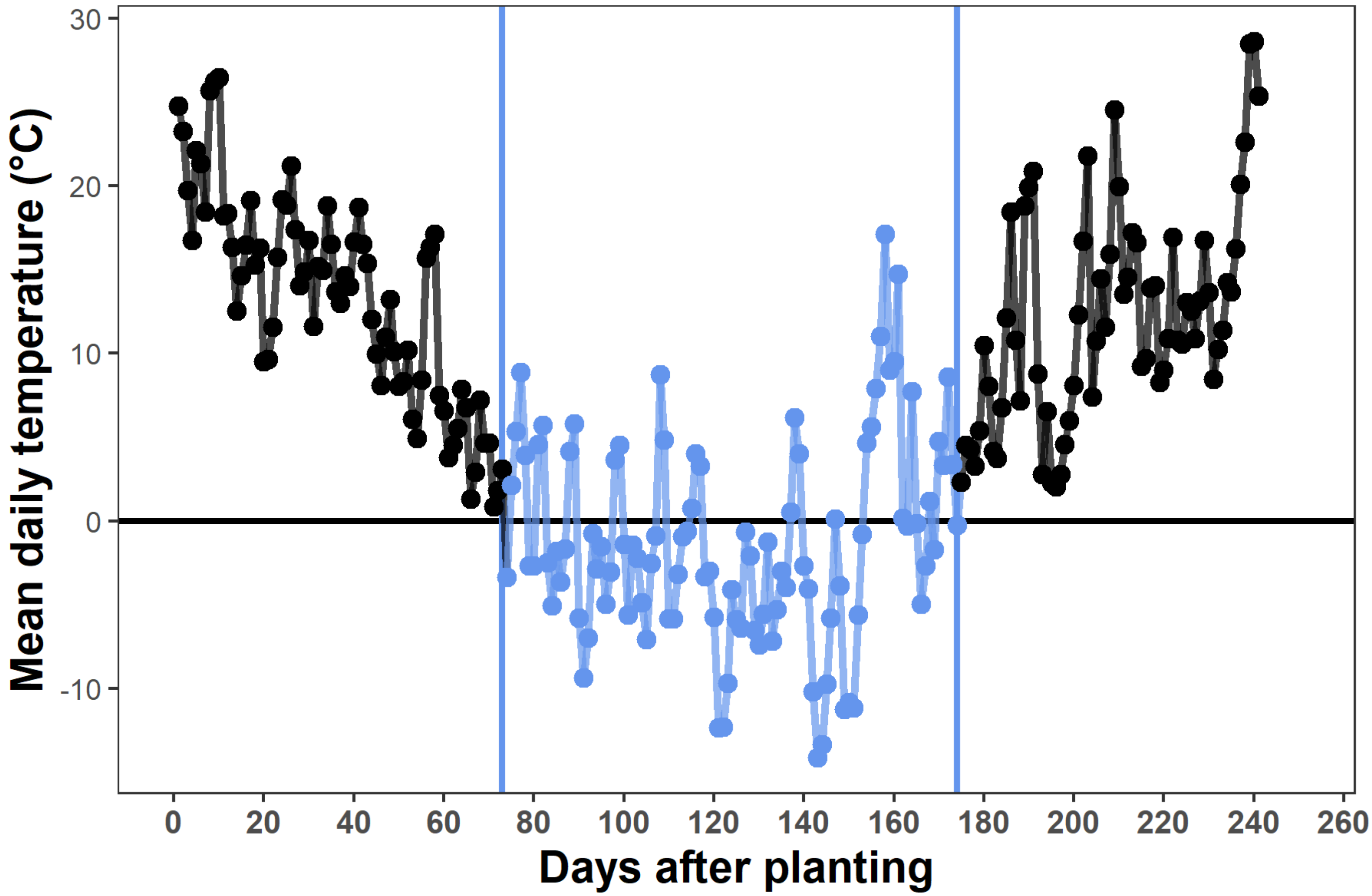


**Figure 2.1.** Example of the cold period. Mean daily temperature (°C) since planting to harvest at Manhattan, KS (2010-2011 season). Blue points represent the cold period. Vertical blue bars represent the beginning and end of cold period as the first and last time that mean daily temperature was below 0 °C, respectively.

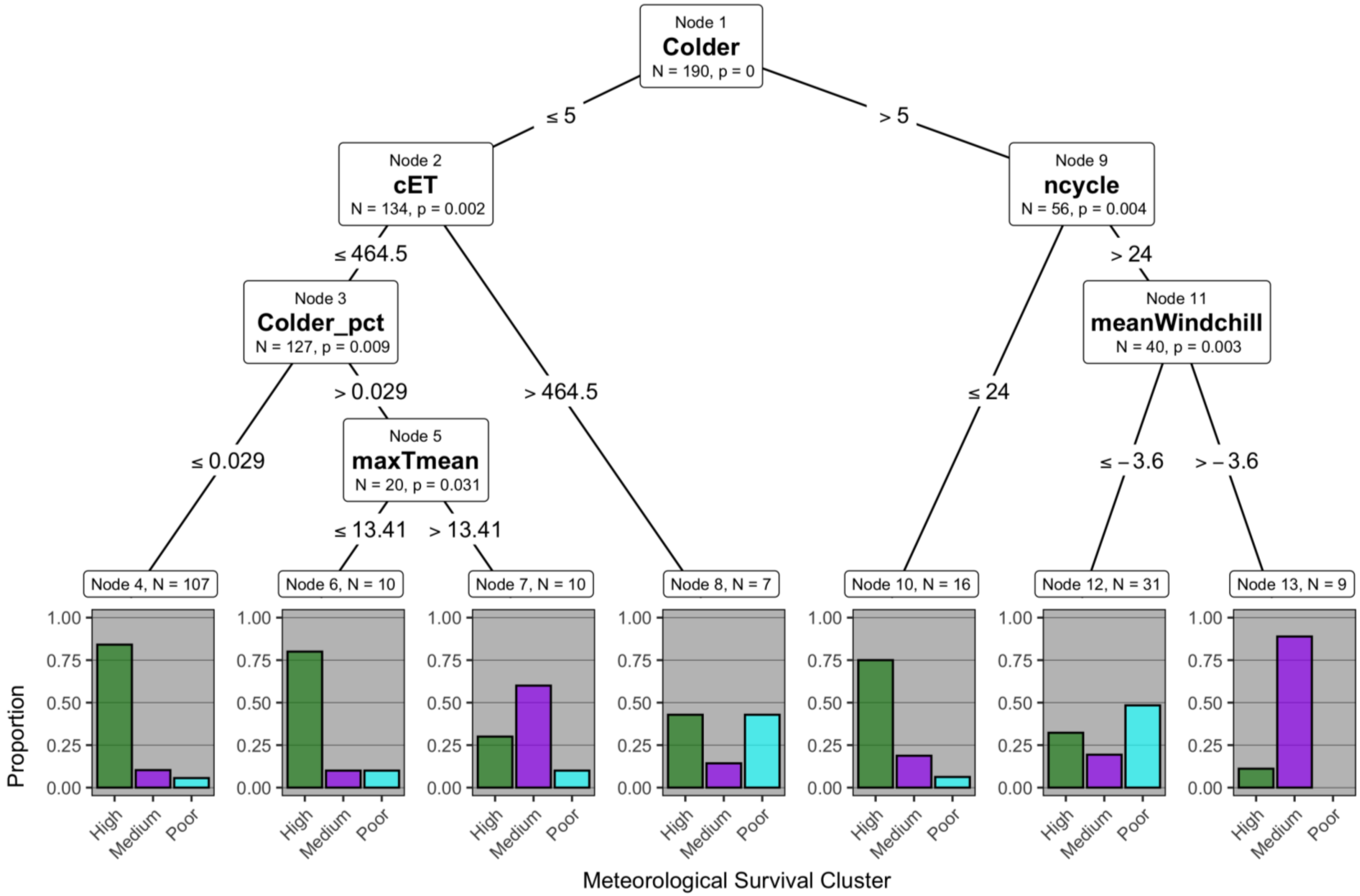


**Figure 2.2.** Conditional inference tree of canola meteorological survival clusters as explained by cold period-summarized weather variables from 190 sites-years classified into seven terminal nodes. Terminal node bars represent the proportion (left y-axis) of site-years within each environmental survival cluster (from left to right at each node: high, medium, and poor) at that node.

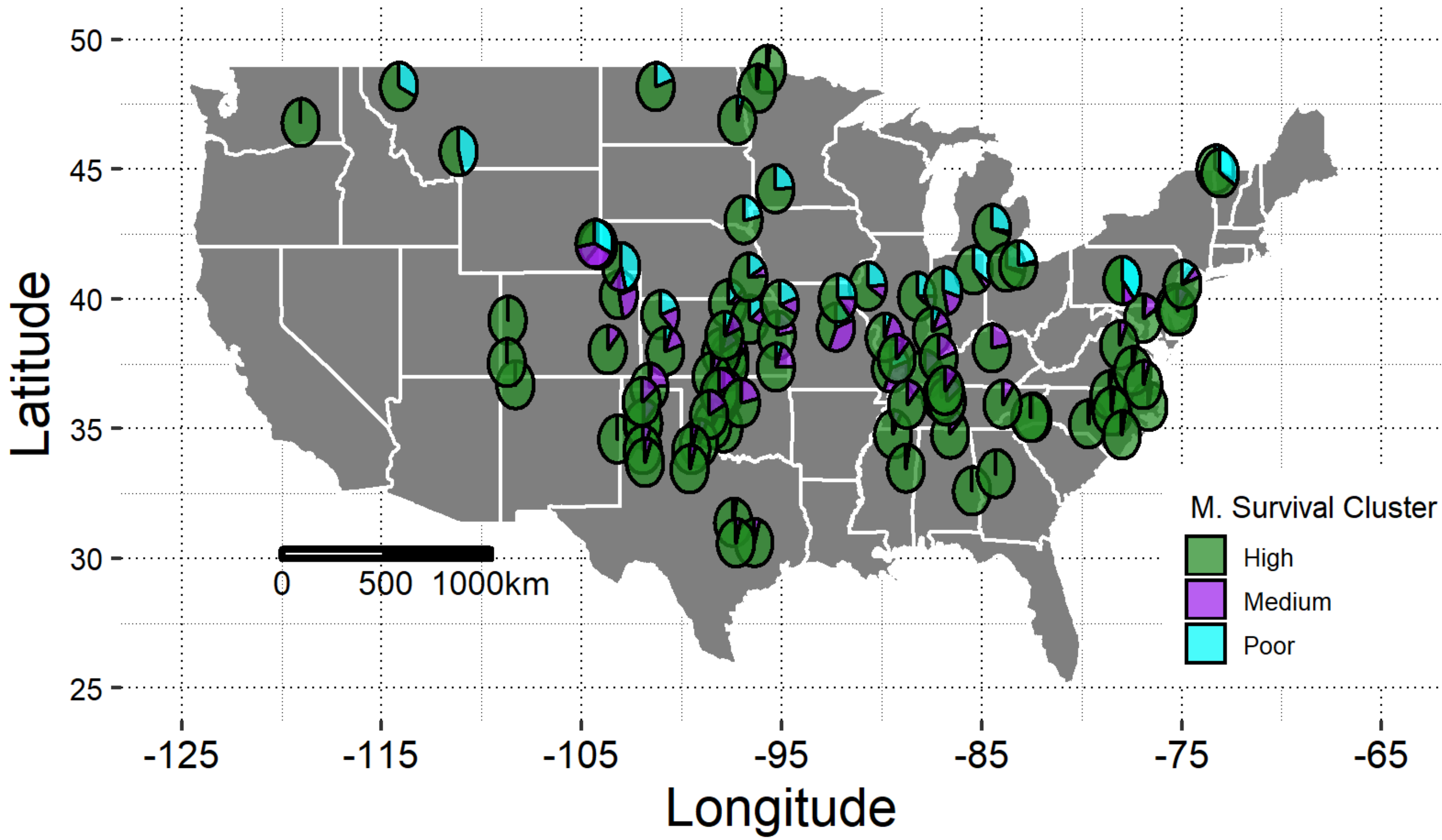


**Figure 2.3.** US map with the sites included in the National Winter Canola Variety Trial dataset. Each pie chart represents a site with the slices representing the proportion of cold periods classified as high, medium, and poor meteorological survival clusters over a period of 40 years (1979-2019).

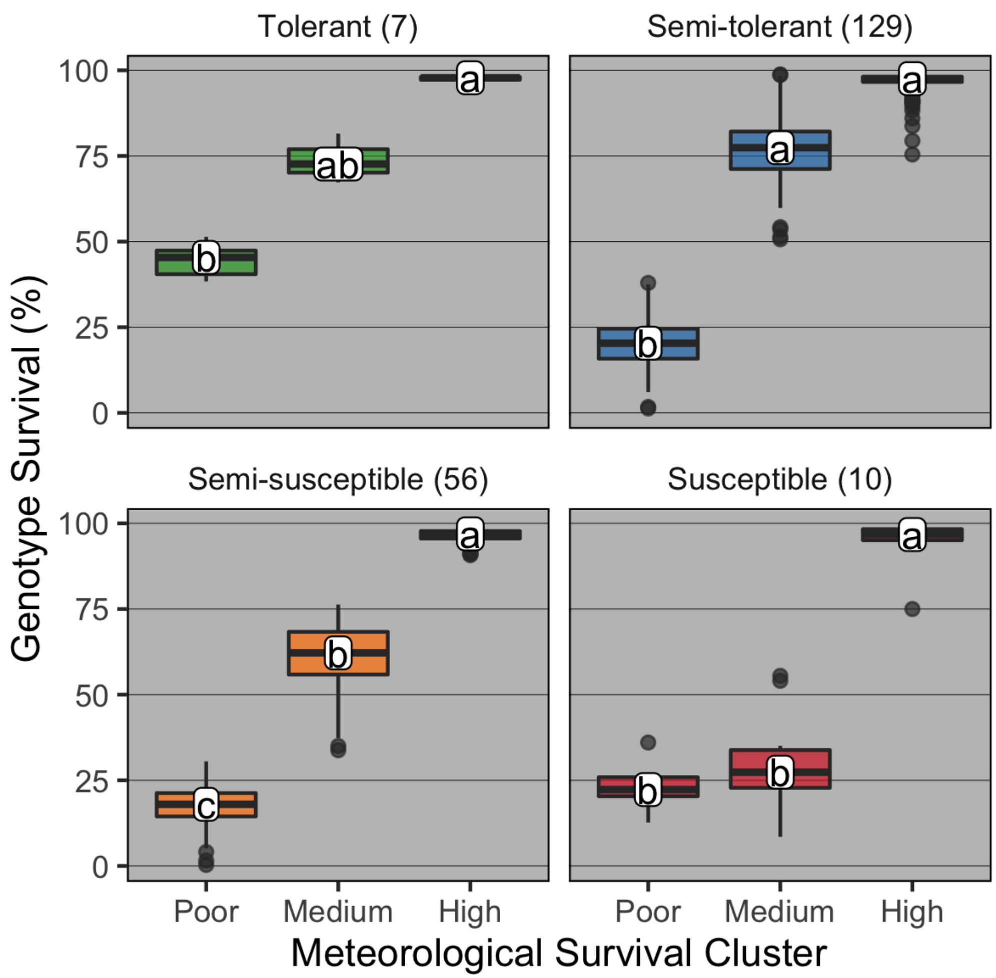


**Figure 2.4.** Boxplots of winter survival of 202 canola genotypes across three different meteorological survival clusters (poor, medium, and high) separated into four distinct genotype survival groups (tolerant, semi-tolerant, semi-susceptible, and susceptible). Boxplots portray the 25th (lower hinge), 50th (solid black line), and 75th (upper hinge) percentiles, largest value no further than 1.5 inter-quartile range (lower whisker), smallest value at most 1.5 inter-quartile range (upper whisker), and outlying observations (points). Boxplots within a panel followed by the same letter are not statistically different at alpha=0.05. Total number of genotypes within each panel is shown in parenthesis.

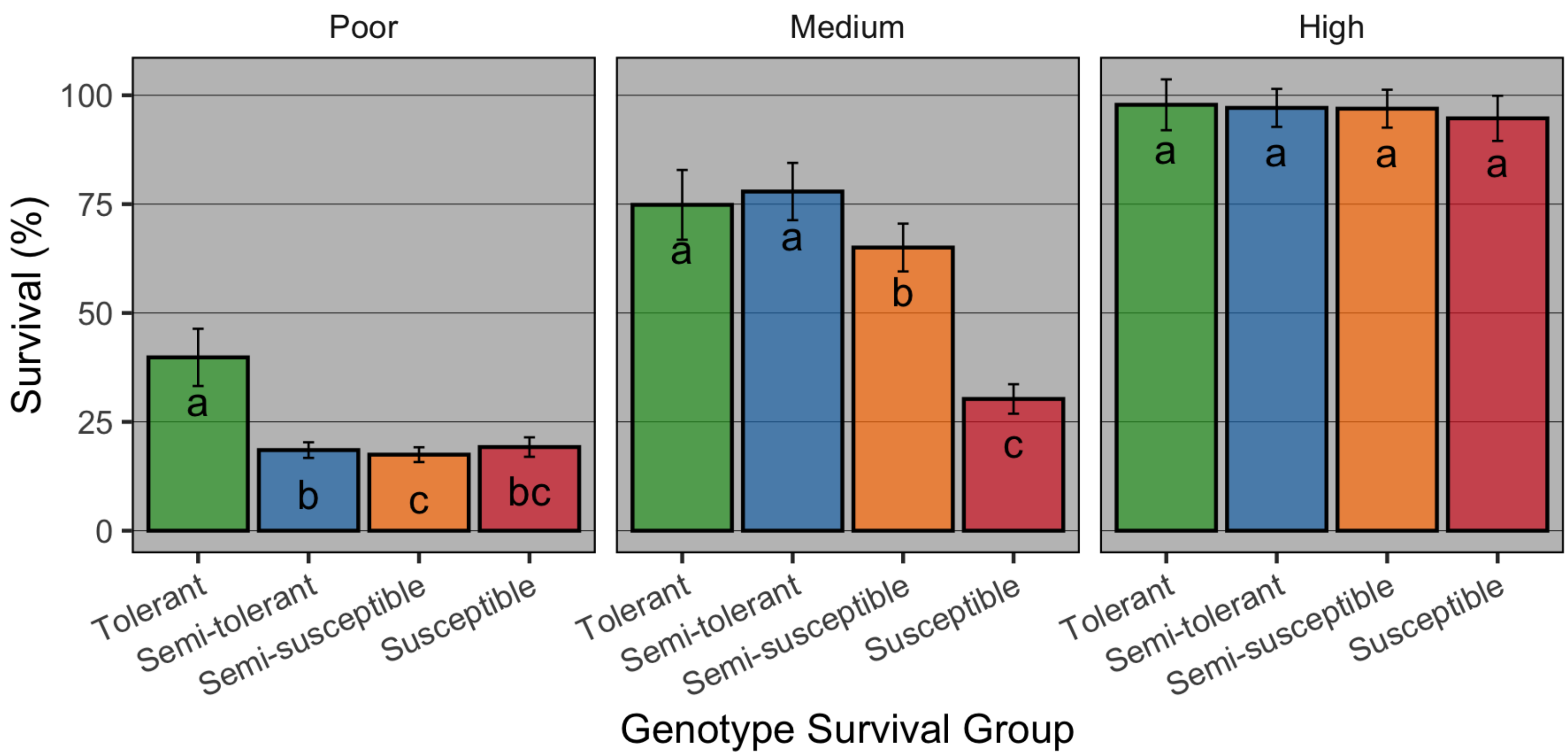


**Figure 2.5.** Canola winter survival means across four different genotype survival groups (tolerant, semi-tolerant, semi-susceptible, and susceptible) for each meteorological survival cluster (poor, medium, high). Means within a panel followed by the same letter are not statistically different at alpha=0.05. Bars represent model-derived standard error.

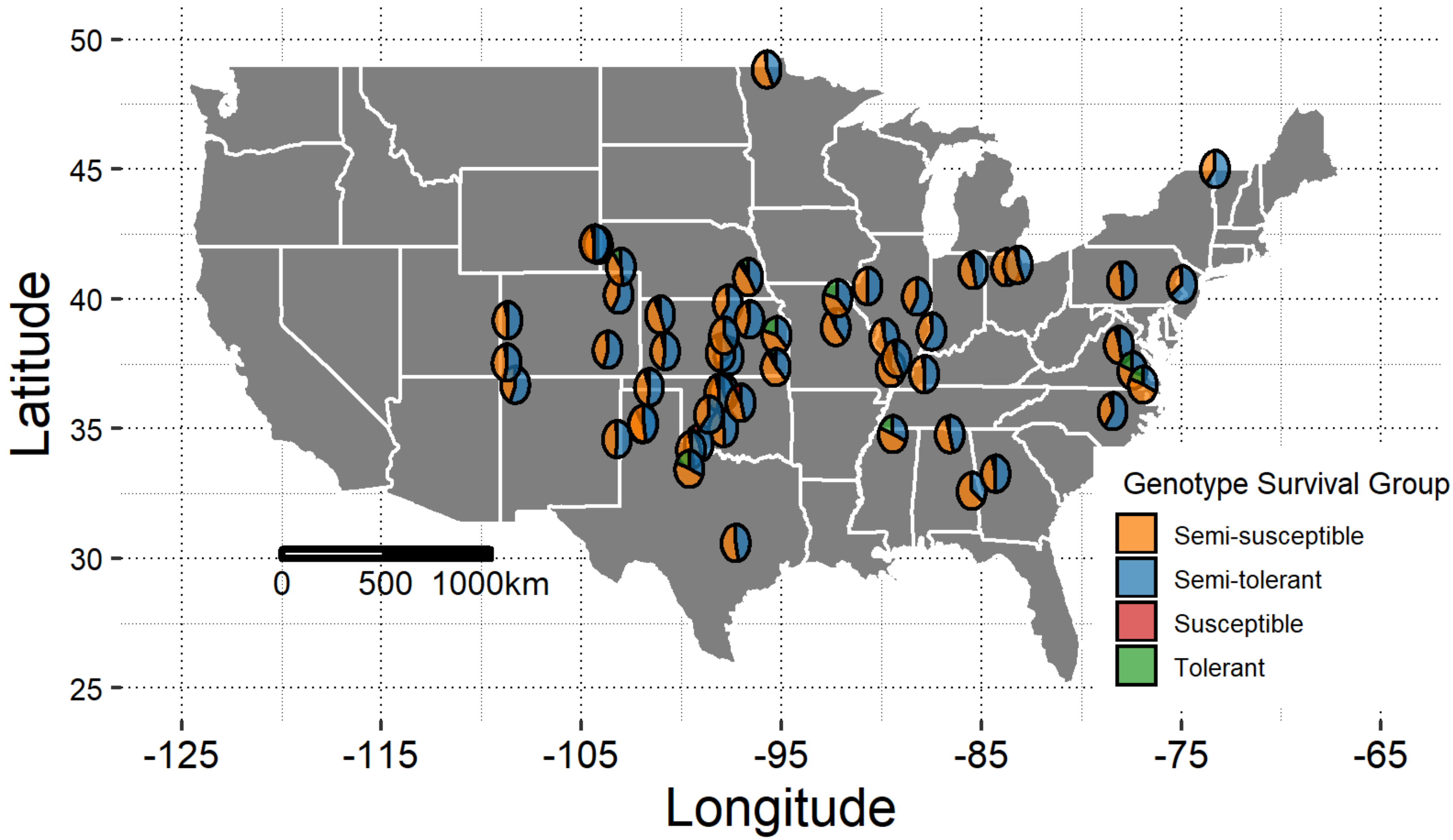


**Figure 2.6.** US map with the sites included in the National Winter Canola Variety Trial, displaying the distribution of each genotype survival group.

**Table 2.1.** Description of secondary daily weather variables.

| Variable | Unit | Abbreviation | Definition |
|---|---|---|---|
| Mean temperature | °C | Tmean | (Tmax + Tmin)/2, |
| Delta temperature | °C | DeltaT | Tmax - Tmin |
| Growing degree days | °C.d. | GDD | (Tmax + Tmin)/2,<br>if Tmax > 30 °C then Tmax=30;<br>if Tmin < 0 °C, then Tmin=0. |

Tmax and Tmin are the maximum and minimum air temperature, in °C, respectively.

**Table 2.2.** Description of meteorological variables during the CP (mean, cumulative, minimum, maximum, and counts).

| Variable | Unit | Abbreviation | Definition |
|---|---|---|---|
| Cold period duration | days | N | Number of days between beginning and end of cold period |
| Number of temperature cycles | count | ncycle | Number of times Tmean shifts from (-) to (+) or vice-versa |
| Slope | °C | - | Slope between cumulative GDD vs. days after planting |
| Tmean descriptors* | | | |
| Warmest, Warmest_pct | count, % | | Number of times Tmean >= 5 °C, Warmest/N |
| Warm, Warm_pct | count, % | | Number of times 0 °C <= Tmean < 5 °C, Warm/N |
| Mild, Mild_pct | count, % | | Number of times -5 °C <= Tmean < 0 °C, Mild/N |
| Cold, Cold_pct | count, % | | Number of times -10 °C <= Tmean < -5 °C, Cold/N |
| Colder, Cold_pct | count, % | | Number of times -15 °C <= Tmean < -10 °C, Colder/N |
| Coldest, Coldest_pct | count, % | | Number of times Tmean < -15 °C, Coldest/N |
| DeltaT descriptors* | | | |
| Extreme, Extreme_pct | count, % | | Number of times DeltaT >= 16.7 °C, Extreme/N |
| High, High_pct | count, % | | Number of times 13.4 °C <= DeltaT < 16.7 °C, High/N |
| Medium, Medium_pct | count, % | | Number of times 10 °C <= DeltaT < 13.4 °C, Medium/N |
| Low, Low_pct | count, % | | Number of times DeltaT < 10 °, Low/N |
| Minimum daily mean temperature | °C | minTmean | - |
| Mean daily mean temperature | °C | meanTmean | - |
| Maximum daily mean temperature | °C | maxTmean | - |
| Minimum daily minimum temperature | °C | minTmin | - |
| Maximum daily maximum temperature | °C | maxTmax | - |
| Minimum delta temperature | °C | minDeltaT | - |
| Mean delta temperature | °C | meanDeltaT | - |
| Maximum delta temperature | °C | maxDeltaT | - |
| Mean wind velocity at 10 m | $m\ s^{-1}$ | meanWind | - |
| Mean wind chill# | °C | meanWindchill | $13.12 + 0.6215\times Tmean - 11.37\times W^{0.16} + 0.3965\times Tmean\times W^{0.16}$ |
| Cumulative precipitation | mm | cPrecip | - |
| Cumulative reference (alfalfa) evapotranspiration | mm | cET | - |
| Cumulative solar radiation | $W\ m^{-2}$ | cSolar | - |
| Cumulative vapor pressure deficit | kPa | cVPD | - |
| Cumulative canola growing degree days | °C.d. | cGDD | SUM (Tmax +Tmin)/2 |

Tmean is the average between maximum and minimum daily temperature, in °C. DeltaT is the difference between maximum and minimum daily temperature, in °C. Tmean and DeltaT descriptors were calculated both as the number of days during winter within a given conditional statement, and as a percentage of this count in relation to total days of winter duration. W is the daily averaged wind velocity at 10 m, in $km\ h^{-1}$

**Table 2.3.** Summary statistics for meteorological variables during the cold period.

| Variable | Unit | Minimum | Mean | Median | Maximum | CV (%) |
|---|---|---|---|---|---|---|
| Survival | % | 0 | 83.9 | 96.2 | 100 | 31.4 |
| N | days | 11 | 111.4 | 109 | 257 | 31.8 |
| ncycle | count | 5 | 23.3 | 23 | 61 | 39.2 |
| Slope | °C | 0.2 | 3 | 2.6 | 10.5 | 64.4 |
| Warmest | count | 0 | 28.9 | 28 | 106 | 57 |
| Warmest_pct | % | 0 | 0.3 | 0.3 | 0.7 | 59.1 |
| Warm | count | 2 | 32.9 | 30 | 100 | 41.7 |
| Warm_pct | % | 0.1 | 0.3 | 0.3 | 0.5 | 27.6 |
| Mild | count | 3 | 31.5 | 32 | 75 | 46.2 |
| Mild_pct | % | 0.1 | 0.3 | 0.3 | 0.5 | 36 |
| Cold | count | 0 | 12.9 | 11 | 47 | 73.7 |
| Cold_pct | % | 0 | 0.1 | 0.1 | 0.3 | 70.7 |
| Colder | count | 0 | 4.1 | 3 | 33 | 125 |
| Colder_pct | % | 0 | 0 | 0 | 0.2 | 172.8 |
| Coldest | count | 0 | 1.2 | 0 | 25 | 266 |
| Coldest_pct | % | 0 | 0 | 0 | 0.2 | 563.8 |
| Extreme | count | 0 | 21.2 | 12 | 86 | 103 |
| Extreme_pct | % | 0 | 0.2 | 0.1 | 0.7 | 101.1 |
| High | count | 2 | 25.5 | 22 | 75 | 62.1 |
| High_pct | % | 0 | 0.2 | 0.2 | 0.5 | 47.8 |
| Medium | count | 1 | 29.8 | 28 | 76 | 46.6 |
| Medium_pct | % | 0 | 0.3 | 0.3 | 0.5 | 36.5 |
| Low | count | 0 | 34.9 | 27 | 130 | 79.7 |
| Low_pct | % | 0 | 0.3 | 0.3 | 0.8 | 66.4 |
| minTmean | °C | -24.9 | -12.6 | -12.6 | -2.3 | -37.7 |
| meanTmean | °C | -7.2 | 1.3 | 1 | 9.1 | 220.7 |
| maxTmean | °C | 4.1 | 14.5 | 14.5 | 21.9 | 22.5 |
| minTmin | °C | -31.1 | -18.4 | -18.2 | -6.9 | -27.2 |
| maxTmax | °C | 6.6 | 22.7 | 22.5 | 34.5 | 17.9 |
| minDeltaT | °C | 0.5 | 4.4 | 4.3 | 12.4 | 46.3 |
| meanDeltaT | °C | 7.1 | 12.5 | 12.5 | 19.1 | 21.1 |
| maxDeltaT | °C | 15.1 | 22.3 | 21.5 | 33.9 | 17.7 |
| meanWind | m s-1 | 2.5 | 4.2 | 4.3 | 5.9 | 13.9 |
| meanWindchill | °C | -12.9 | -2.5 | -2.7 | 7.5 | -138.6 |
| cPrecip | mm | 1.8 | 149 | 107.6 | 608.7 | 82.3 |
| cET | mm | 29.9 | 270.6 | 245.7 | 701.3 | 44 |
| cSolar | W m-2 | 1424 | 12479 | 11736 | 27765 | 36.5 |
| cVPD | kPa | 4.1 | 40.6 | 35.8 | 96 | 47 |
| cGDD | °C.d. | 13.1 | 333.4 | 307.2 | 1161.7 | 53.8 |

CV=coefficient of variation.

# Chapter 3 - Suitability of different environments for winter canola oil production in the United States of America

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## Abstract

Including winter canola (*Brassica napus* L.) into cereal-based crop rotations can diversify the current agricultural systems in the United States (US). In addition, canola can help to satisfy market demand for plant-based edible oil, thus better understanding of oil productivity of canola in US production areas is relevant for further study. Canola seed oil concentration is a function of the genotype (G), environment (E), management (M), and their interaction (G × E × M). The overall aim of this research study was to identify suitable production environments with increased canola seed oil concentration and opportunities to increase cropland area in the US. The objectives of this research were to i) characterize the environmental variability of seed oil concentration in the National Winter Canola Variety Trials (NWCVT), ii) identify geographical regions and states within the continental US with potential for production of winter canola with high seed oil concentration and the link with yield, and iii) explore the potential development of varieties with stable high seed oil concentration and high yield. In this study, the E component accounted for 75% of the total variation in seed oil concentration, with the G factor only explaining 8%. Overall, seed oil concentration averaged 398 g kg$^{-1}$ but ranged from 311 to 461 g kg$^{-1}$. The US Midwest and Great Plains regions were shown to have a greater frequency of medium (> 389 g kg$^{-1}$) and high canola seed oil concentration (> 411 g kg$^{-1}$). Genotypic variation for both seed oil concentration and yield was found with an opportunity to achieve high oil and yield under varying environments. Our findings suggest that there is significant potential to

further expand the area of canola cropland to increase oil productivity and focus should be on breeding high yielding varieties with stable, high seed oil concentration.

## 3.1 Introduction

Canola (*Brassica napus* L.) is a rapeseed cultivar with edible oil quality. Among several oilseed crops, rapeseed has a worldwide estimated total production of 73 million tonnes, ranking as the second most important oilseed crop after soybeans (*Glycine max* L.) (FAOSTAT, 2022). From the standpoint of US production, the area sown to canola has increased from 60 to about 740 thousand hectares from early 1990's to 2021 period (USDA-ERS, 2021), mainly due to sowing of well adapted Canadian spring types in the northern Plains. Winter canola hectares in the Great Plains declined in the last few years due to the loss of productivity and other farming challenges (USDA-NASS, 2021). Consistency of yield and oil concentration are the two most relevant factors directly affecting economic farm decisions which led to this decline.

Better understanding of the environmental drivers for oil production and optimum selection of canola varieties will assist in providing new opportunities to expand the geographical area, overall production, increase crop diversification, and providing farmers with options for crop rotation. For example, including canola into wheat-based (*Triticum aestivum* L.) farming systems, not only reduced the impacts of diseases and weeds but most importantly improved the overall profit of the system (Bushong et al., 2012). As a broadleaf crop, canola provides an opportunity for using more effective and less expensive herbicides to control grassy weeds, relative to cereal-based farming systems (Zollinger, 2013). The future of winter canola expansion depends on identifying optimal genotype, environment, and management (G x E x M) combinations for both yield and seed quality.

Seed oil concentration is the critical seed quality attribute which positions winter canola as one of the best crop options to satisfy growing oil and biofuel demands. In other major canola growing areas across the globe, the average seed oil concentration typically ranges from 450 to 500 g $kg^{-1}$ in Europe and from 360 to 450 g $kg^{-1}$ in southern Australia (Gunasekera et al., 2006; Wittkop et al., 2009). In the US, seed oil concentration ranges from 300 to 500 g $kg^{-1}$ and with most of the variation attributed to the environmental conditions (79%) during the crop growth (Assefa et al., 2014). Nonetheless, the effect of the interaction between different G × E × M on seed oil concentration for US winter canola is unclear. Maximum seed oil concentration is achieved when seeds fill under cooler temperatures (Walton et al., 1999). Between anthesis and pod filling, heat and drought decrease canola yield and oil quality (Gan et al., 2004; Weymann et al., 2015). In the US, in some years, seed filling for winter canola occurs when temperatures are near the maximum for optimum crop development (30ºC). However, the main environmental conditions driving seed oil change are unclear and the extent to which of any G × E interaction. Unfortunately, unfavorable conditions may result in producers receiving price discounts from seed crushers when oil concentrations are at or below critical levels. Thus, enhancing our knowledge of the suitability of environments for US winter canola production will be critical to improving the competitiveness on the global canola market.

The aim of this study was to identify suitable winter canola production environments with a higher seed oil concentration. Specific objectives were to i) characterize the environmental variability of seed oil concentration, ii) identify geographical regions and states within the continental US suited to winter canola production with both high seed oil concentration and yield, and iii) explore the potential development of varieties with stable and high seed oil concentration and yield.

## 3.2 Materials and Methods

### Data curation

#### *Field dataset and response variables*

The field dataset was curated from the National Winter Canola Variety Trial (NWCVT) (Stamm et al., 2019). The purpose of this national network of trials is to evaluate yield performance and other important agronomic traits such as seed oil concentration across many US states. The entire field dataset comprises 25 sites covering 13 US states, spanning from 2003 through 2018, and includes a total of 288 varieties. Only site-years with seed yield, oil concentration, flowering date, and maturity date information were included in this study (56 site-years). The experimental design for each trial defined by site and year was a randomized complete block with three or four replications, depending on farm collaborator specifications. Seed oil concentration was measured using near-infrared (NIR) spectroscopy or nuclear magnetic resonance (NMR), reported on a percentage (%) basis, and later converted to concentration (g $kg^{-1}$).

***Weather data***

Daily weather data were extracted from Google Earth Engine (Gorelick et al., 2017) for the period 1979-2019 (41 years) for each site using the latitude-longitude coordinates. The data set included daily minimum (Tmin) and maximum (Tmax) temperatures (°C), precipitation (mm), and solar radiation (W $m^{-2}$). The daily weather data was utilized to i) create 5 secondary weather variables to correlate with seed oil concentration (Table 3.1) on three phases of the seed filling period (explained later under the data processing section); ii) build a model to predict seed oil concentration clusters in relation to environmental variables; and iii) predict changes in seed oil concentration for different environments and calculate the frequency of this seed quality trait for each environment over 41 years. Secondary environmental variables were calculated to capture meteorological differences for each phase. Specifically, the photothermal quotient (PTQ) as the ratio between cumulative solar radiation and cumulative growing degree days (cGDD). The Shannon Diversity Index (SDI) was calculated (Bronikowski & Webb, 1996) with the function diversity from the R-package *vegan* (Oksanen et al., 2020) to characterize the distribution of the precipitation during each phase. The cGDD was calculated, first for the entire period to describe the crop development and later, to divide the seed filling period into three phases for separate analysis.

***Weather during seed filling period***

From the field dataset for each site-year, flowering and maturity dates were averaged over all varieties and used as the earliest and latest limits of the seed filling period. The seed filling period boundaries were used to filter the weather dataset for each site-year and then to calculate the cGDD. At each site-year, we further divided the seed filling period into three phases of equal thermal duration (cGDD) in (i) early, (ii) medium, and (iii) late (herein termed as Phase 1, Phase 2, Phase 3, respectively). The seed filling period across site-years had a wide range of cGDD from 639 to 1448 °C.d. There was wide variation in the environmental parameters (Table 3.2) since the dataset contained sites from northern Montana to southern Texas and from western New Mexico to Vermont.

## Data analysis

***Descriptive***

Both seed oil concentration and yield values were summarized with descriptive statistics mean, interquartile range $IQR_{25\text{-}75}$ (between 25$^{th}$ and 75$^{th}$ percentiles), and observations were classified based on the empirical distributions in (i) Low, below the 33$^{rd}$ percentile, (ii) Medium, between the 33$^{rd}$ and the 66$^{th}$ percentiles, and (iii) High, above the 66$^{th}$ percentile.

***Analysis of oil G × E variance***

A first model using the seed oil concentration (n = 4,567) as the response variable was estimated using a random-effects structure with G, site-year (E), and their interaction (G × E) to retrieve the proportion of the oil variance explained by (G × E) components.

### *Oil environment classification*

A second model aimed to elucidate variables describing environments (site-years) that favored from Low to High seed oil concentrations in canola. For this purpose, a simple classification tree algorithm (Breiman et al., 1984) was used.

The response variable was the oil concentration class (categorical), with three levels: Low (< 389 g $kg^{-1}$), Medium (389 g $kg^{-1}$ to 411 g $kg^{-1}$), and High (> 411 g $kg^{-1}$). The classification of oil concentration was performed at the site-year level (n = 56), averaging all varieties present in each case.

As predictors of the model, 15 meteorological variables were used, resulting from the combination of the five environmental variables (Table 3.2) at three phases during the seed filling period (Phases 1, 2, and 3). To prune the tree, the complexity parameter (cp) was set to a minimum improvement rate of 0.05 to find an adequate compromise between the misclassification rate and the tree size. To evaluate the classification tree performance, the accuracy was calculated as the proportion of observations well-classified at each tree node.

### *Oil environment probability map*

By applying the final classification tree rules, a series of 41 years (1979-2019) of environmental conditions during the seed filling period were used to estimate the frequency of the seed oil concentration environments (Low, Medium, and High) at each of the 25 locations.

### *Genotype performance by oil environment*

In order to study the G × E interaction effects on oil productivity, only a subset of canola varieties (n = 43) was retained using the following criterion: (i) tested in more than 10 site-years, and (ii) tested under variable weather conditions during the seed filling period that, according to the classification tree, would favor Low, Medium and High oil concentration environments, at least once each. Within each expected oil concentration environments (Low, Medium or High), the performance of varieties was compared using the average oil concentration across site-years. Performance was classified as: (i) top-10 (Class I), (ii) top-20 (Class II), and the remaining varieties (Class III). In addition, the top-10 varieties for oil concentration were described in terms of attained yield levels.

***Software and statistical analyses***

All analyses were conducted within the R software framework (R Core Team, 2019). Mixed model analyses were run using the function *lmer* from the *lme4* package (Bates et al., 2015b). The classification tree model was performed using the function *rpart* (Hothorn et al., 2006) from the *rpart* package (Atkinson, 2019).

## 3.3 Results

***Oil and yield summary***

Overall, seed oil concentration averaged 398 g $kg^{-1}$, ranging from 311 to 461 g $kg^{-1}$; and seed yield averaged 2.6 Mg $ha^{-1}$, with a range from 0.13 to 5.3 Mg $ha^{-1}$. More than 20% of observations combined Medium ($IQR_{25\text{-}75}$ = 395 - 405 g $kg^{-1}$) and High ($IQR_{25\text{-}75}$ = 416 - 435 g $kg^{-1}$) seed oil concentration with Medium seed yield ($IQR_{25\text{-}75}$ = 2.3-2.7 Mg $ha^{-1}$) (Fig. 3.1). High seed oil concentration with High seed yield was less frequent than High seed oil concentration with Medium seed yield levels.

***Analysis of G × E variance***

The E component accounted for 75% of the total variation in seed oil concentration, whereas the G and the G × E components explained 8% and 3%, respectively. The residual variance resulted at 13%.

***Oil environments classification***

The classification tree resulted in a simple four terminal nodes-model that correctly classified 75%, 69%, and 75% of observations at Low, Medium, and High oil concentration environments, respectively (Figure 3.2). The tree identified that cumulative precipitation was of key importance during Phases 1 and 2, while PTQ was central during Phase 2. The classification tree indicates that environments with at least 35 mm of precipitation will have higher seed oil concentrations. In combination to > 35 mm of rain during Phase 1, a PTQ of less than 1.2 MJ $m^{-2}$ / °C.d. will further enhance the accumulation of seed oil. The final condition to achieve the highest oil concentration would be not to exceed 56 mm of precipitation during the Phase 2 of the seed filling period.

***Oil environment probability map***

The estimated frequency of canola oil concentration across the US sites included in the study (Figure 3.3) indicated a prevalence of low oil concentration environments in the states of Vermont, New Mexico, Colorado, and New Jersey. Medium oil concentration environments resulted more frequent (> 70%) in the states of Montana, Missouri, Mississippi, Virginia, Kansas, Maryland, and Georgia. Overall, high oil concentration environments were infrequent and only one state (Alabama) had high oil concentration environments 40% of the time.

### *Variety performance by oil environment*

From a total of 288 varieties in the database, only 77 winter canola varieties were grown across all oil environments, from which only 43 were tested in more than 10 site-years. Seed oil concentration ranged from 356 to 432 g $kg^{-1}$ (Mean = 383 g $kg^{-1}$; $IQR_{25-75}$ = 358-409 g $kg^{-1}$), 389 to 426 g $kg^{-1}$ (Mean = 407 g $kg^{-1}$; $IQR_{25-75}$ = 398-417 g $kg^{-1}$), and 360 to 439 g $kg^{-1}$ (Mean = 413 g $kg^{-1}$; $IQR_{25-75}$ = 384-435 g $kg^{-1}$) for Low, Medium, and High oil environments, respectively. Four varieties that consistently ranked within the top-10 across the three oil environments were: (i) KS4426, (ii) Hybrirock, (iii) Dimension, and (iv) Dynastie (Figure 3.4). Although these four varieties showed similar and stable oil levels across environments (413-420 g $kg^{-1}$, $CV_{oil}$ = 6.5-7.8%), the first two showed more stable yields when changing the oil environment, from low to high ($CV_{yield}$ = 22-26%), as compared to the latter two ($CV_{yield}$ = 39-42%).

In terms of response to changing oil environment, some varieties exhibited contrasting behavior in terms of both seed oil concentration and seed yield performance. For example, for the Low oil environments, AAMU-33-07 ranked as Class I (top-10), showing an average seed oil concentration of 402 g $kg^{-1}$ and seed yield of 1.98 Mg $ha^{-1}$, while ranked as Class III (below the top-20) in the High oil environments, decreasing seed oil concentration (392 g $kg^{-1}$) with an increase in seed yield (2.39 Mg $ha^{-1}$). In contrast, 46W99 ranked as Class III for the Low oil environments (seed oil concentration of 364 g $kg^{-1}$; and seed yield of 1.63 Mg $ha^{-1}$), while ranked as Class I (top-10) for the High oil environments, increasing both seed oil concentration (426 g $kg^{-1}$) and seed yield (2.79 Mg $ha^{-1}$). The latter example shows, in a nutshell, the genotypic variability that could be still exploited for both seed oil and yield on winter canola, with some varieties showing more oil stability under changes in yield (e.g., AAMU-33-07) and some other ones (e.g., 46W99) showing great responsiveness in both plant traits, but with low levels of those under Low oil environments (Figure 3.4).

## 3.4 Discussion

This synthesis-analysis provides new insights on the variability of winter canola varieties and geographical distribution, with precipitation and PTQ determined to be key weather variables during the seed filling period. In addition, this study assists on providing foundational knowledge for canola breeding programs and seed suppliers to select varieties targeting medium or high oil levels with high yield in the US. To the extent of our knowledge, this study provides the first analysis of spatio-temporal variability and its drivers for winter canola seed yield and oil concentration. Past studies on spatial characterization have been mainly focused on other crops such as soybeans (*Glycine max* (L.) Merr.) (Assefa et al., 2018; Rotundo et al., 2016), sorghum (*Sorghum bicolor* L.) (U.S. Grains Council, 2017), corn (*Zea mays* L.) (US Grains Council, 2020) but scarce information is available for US winter canola (Assefa et al., 2014).

The environment is a key factor driving the seed oil composition for canola (Gunasekera et al., 2006; Liersch et al., 2020; Pritchard et al., 2000; Si et al., 2003), as indicated by our findings. Nonetheless, the present study goes a step further by (i) identifying environmental drivers, and (ii) inter-annual weather conditions affecting seed oil concentrations. Winter canola has been promoted in US states such as Indiana and Michigan (Christmas & Hawkins, 1992; Copeland et al., 2001) and regions such as the Midwest, Great Plain (Assefa et al., 2014), and Pacific Northwest (J. Brown et al., 2008; F. L. Young et al., 2014). Our results indicate similar proportions of medium and high oil concentration in the Midwest relative to the Northwest region.

The main weather drivers affecting seed oil concentration have been less explored than those influencing yield formation, with water availability (Champolivier & Merrien, 1996; Farré et al., 2001; Jensen et al., 1996) and high temperature (Aksouh-Harradj et al., 2006; Elferjani & Soolanayakanahally, 2018; Faraji et al., 2009; Lohani et al., 2021; Pokharel et al., 2021) identified as key factors. Precipitation during the seed filling period affects water availability and has been reported to be positively correlated with seed oil concentration (Pritchard et al., 2000; Si et al., 1999; Walton et al., 1999, Si et al., 2003). For example, several studies have shown a reduction in seed oil concentration under water deficit after flowering (Aslam et al., 2009; Champolivier & Merrien, 1996; Jensen et al., 1996; Pritchard et al., 2000). One potential explanation is that water deficit results in plants closing their stomata, thus limiting their ability to photosynthesize take up and fix carbon dioxide for eventual production of oil. Studies of the effect of heat stress on seed oil concentration are inconsistent, with positive or neutral effects (Pokharel et al., 2021) as well as negative effects (Elferjani & Soolanayakanahally, 2018; Hocking & Mason, 1993; Pritchard et al., 2000) reported. The PTQ during the seed filling was positively correlated with seed oil concentration (Faraji, 2012), but in our study, PTQ was positively correlated for medium oil levels only, with high oil levels potentially more impacted with high seed yields and lower oil levels.

From a plant breeding perspective, identifying stability for both yield and oil performance under a large range of environments is crucial (Moghaddam and Pourdad, 2011; Turhan et al., 2011; Guo et al., 2017). In our study, we identified varieties with stable performance for both seed oil concentration and yield (Figure 3.4). AAMU-33-07 ranked in Class I (top-10) for oil production in Low oil environments, but Class III in High oil environments. This open-pollinated cultivar was developed for southeastern US environments, for early flowering and maturity to outpace spring heat in the region, as a stress avoidance mechanism. In several other cases, varieties showed contrasting oil and yield performance depending on the environment. 46W99 (developed outside of the US) ranked as Class III for the Low oil environments while ranking as Class I (top-10) for the High oil environments. This hybrid has shown extremely high yield potential in low-stress environments (Stamm et al., 2019). Adaptability outside the US could explain the contrasting performance across diverse environmental conditions. In addition, KS4426, an open-pollinated experimental cultivar (Stamm et al., 2019), consistently ranked within the top-10 across all oil environments and showed stable yields. Lastly, in general, breeding programs face the challenge to further exploit G × E to develop either specific varieties adapted to local regions (Turhan et al., 2011) or varieties with greater performance stability across wide geographical regions (Guo et al., 2017).

The current study attempts to emphasize the need for pursuing both high seed oil and yield using long-term multi-environment trials (METs). Likewise, Zhang et al. (2013) and Turhan et al. (2011), targeted canola breeding efforts for specific genotype adaptation to mega-environments or regions. Initial efforts exploring the G × E for both seed yield and oil concentration are reported in countries such as Australia, Turkey, China, and Poland, (Beeck et al., 2010; Cullis et al., 2010; Guo et al., 2017; Liersch et al., 2020; Niemann et al., 2018; Turhan et al., 2011; Zhang et al., 2013). However, most of the focus of the G × E interaction has been explored in METs mainly for seed yield (Alizadeh et al., 2021; Marjanović-Jeromela et al., 2011; Morrison et al., 2016; Puhl et al., 2019).

One limitation of this work is that not all varieties were tested over all sites, reducing the power to understand the contribution of the G component to the variation in seed oil concentration at spatial-temporal scales. In addition, detailed physiological research on impact of environmental factors and their interactions for both oil and yield on canola seed filling phases is needed. Investing resources to understand the physiological processes underpinning this complex seed quality trait (with yield) and its interaction with other G × E × M will be needed. From the farming decision, remanent challenges are linked to stand establishment, winter survival, silique shattering, harvesting, and proximity of seed delivery point (Stamm & Watson, 2013).

## 3.5 Conclusions

Analysis of the US winter canola trials showed that most of the variability in seed oil concentration could be explained the Environment (site-year). Precipitation during Phases 1 and 2 of the seed filling period and the PTQ during Phase 2 were the most relevant environmental variables explaining winter canola seed oil concentration. This study demonstrates that there is a large geographical area with potential for medium and high canola seed oil concentration in the US southeastern region. Genotypic variation for both seed oil and yield was found, indicating an opportunity to develop varieties with high seed oil concentration and yield. This work provides foundational knowledge for canola breeding programs to target medium or high oil levels and high seed yield.

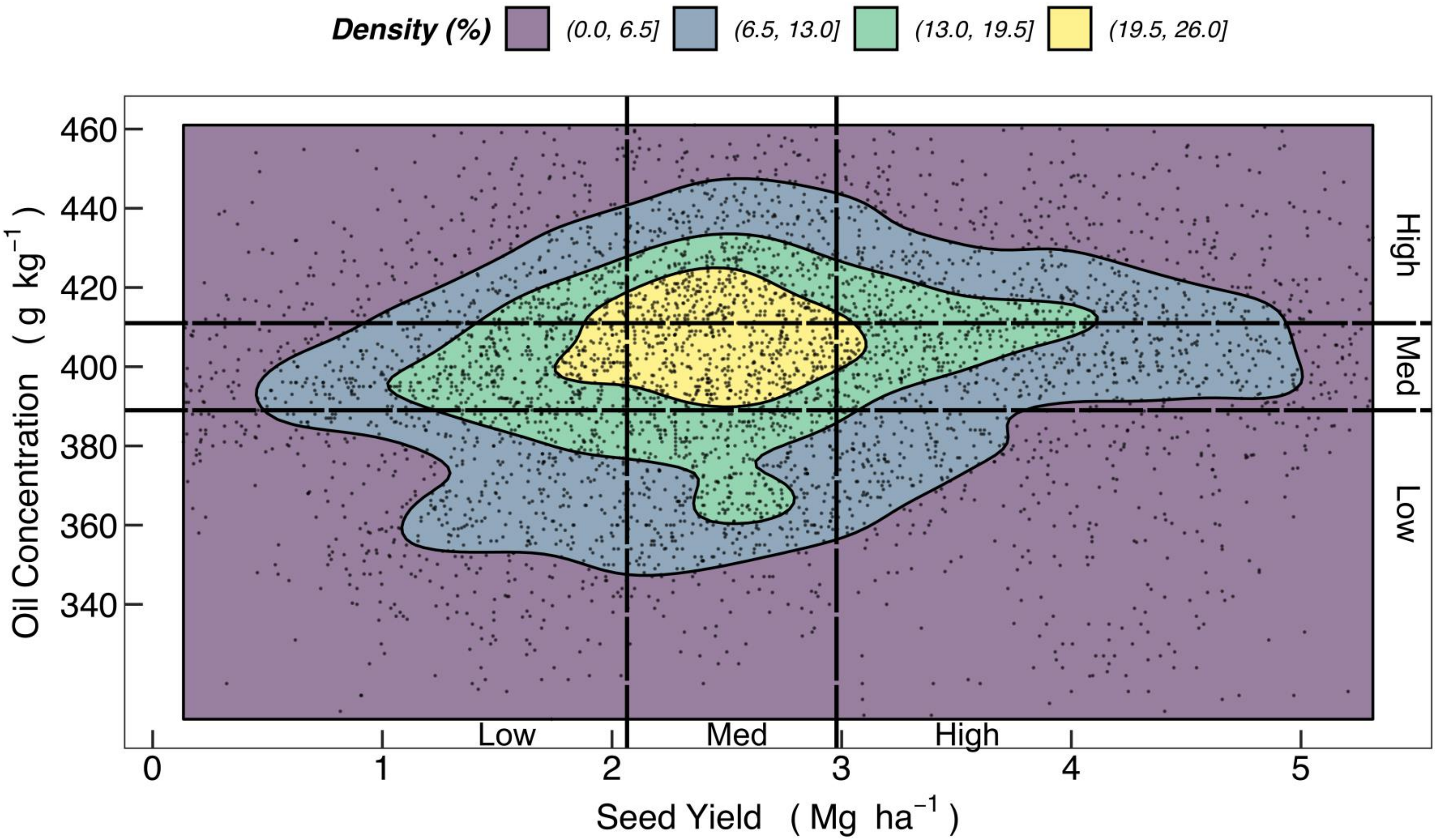


**Figure 3.1.** Joint density distribution of seed oil concentration (expressed in g $kg^{-1}$) along with seed yield (Mg $ha^{-1}$) for winter canola (n=4,567) observations from the National Canola Winter Variety Trial dataset, 25 sites covering 13 US states (2003-2018) with 288 varieties and a total of 56 site-years. Different colors represent different groups with observations density range (%). Dashes lines represent the 33$^{rd}$ and 66$^{th}$ percentiles for both axes identifying Low, Medium (Med), and High levels.

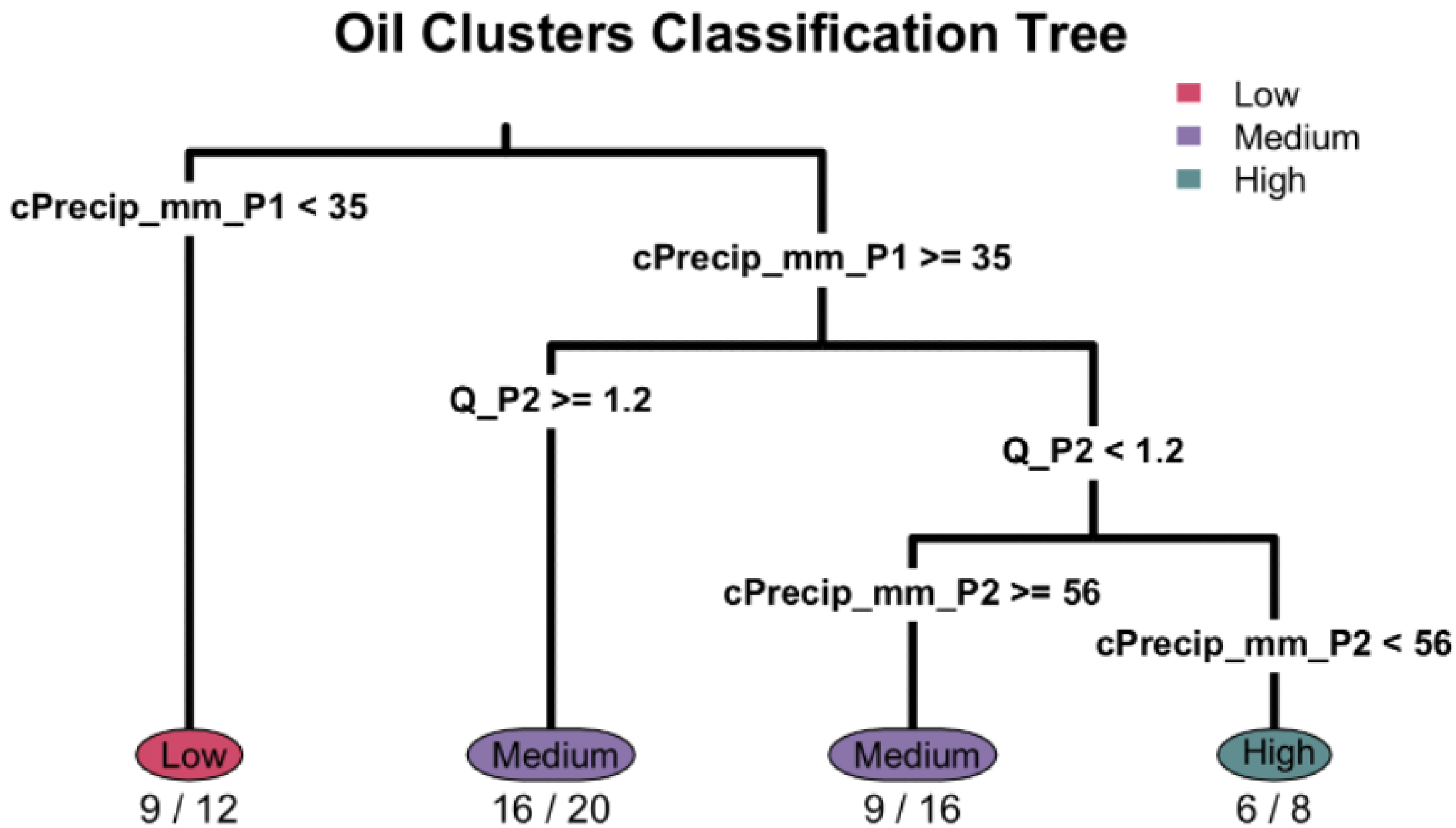


**Figure 3.2.** Winter canola oil concentration clusters conditional inference tree explained by summarized meteorological variables in three seed filling phases. cPrecip_mm_P1 = Cumulative precipitation during phase 1, PTQ_P2 = Photothermal quotient during phase 2, cPrecip_mm_P2 = Cumulative precipitation during phase 2. Fractions below the final nodes represent the accuracy of each node.

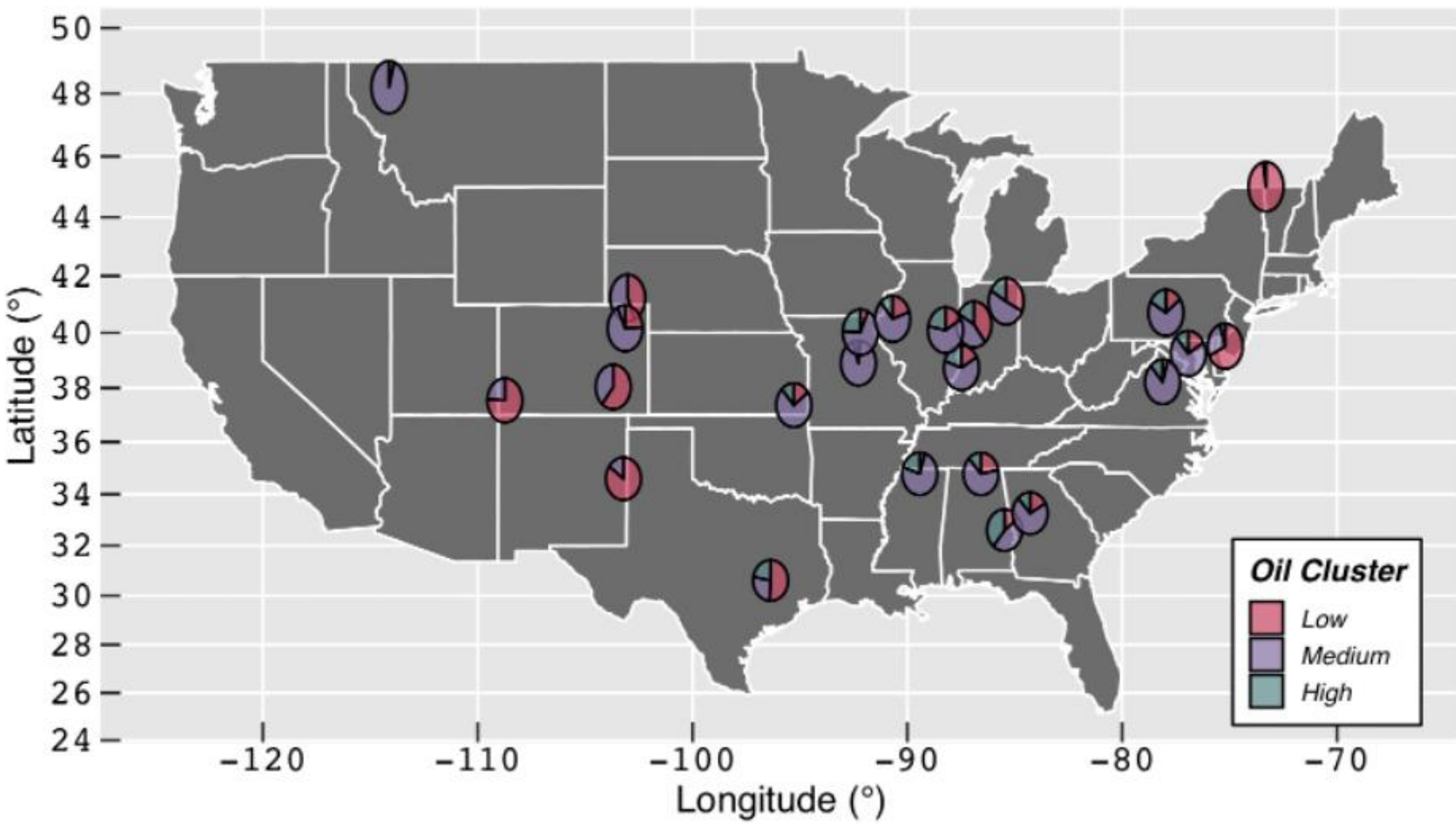


**Figure 3.3.** Continental United States (US) sites included in the National Winter Canola Variety Trial dataset. Each pie chart depicts a site with proportion of years when the model classified as Low, Medium, and High seed oil concentration clusters (herein termed as Oil Cluster) over a period of 41 years of historical weather data (1979-2019).

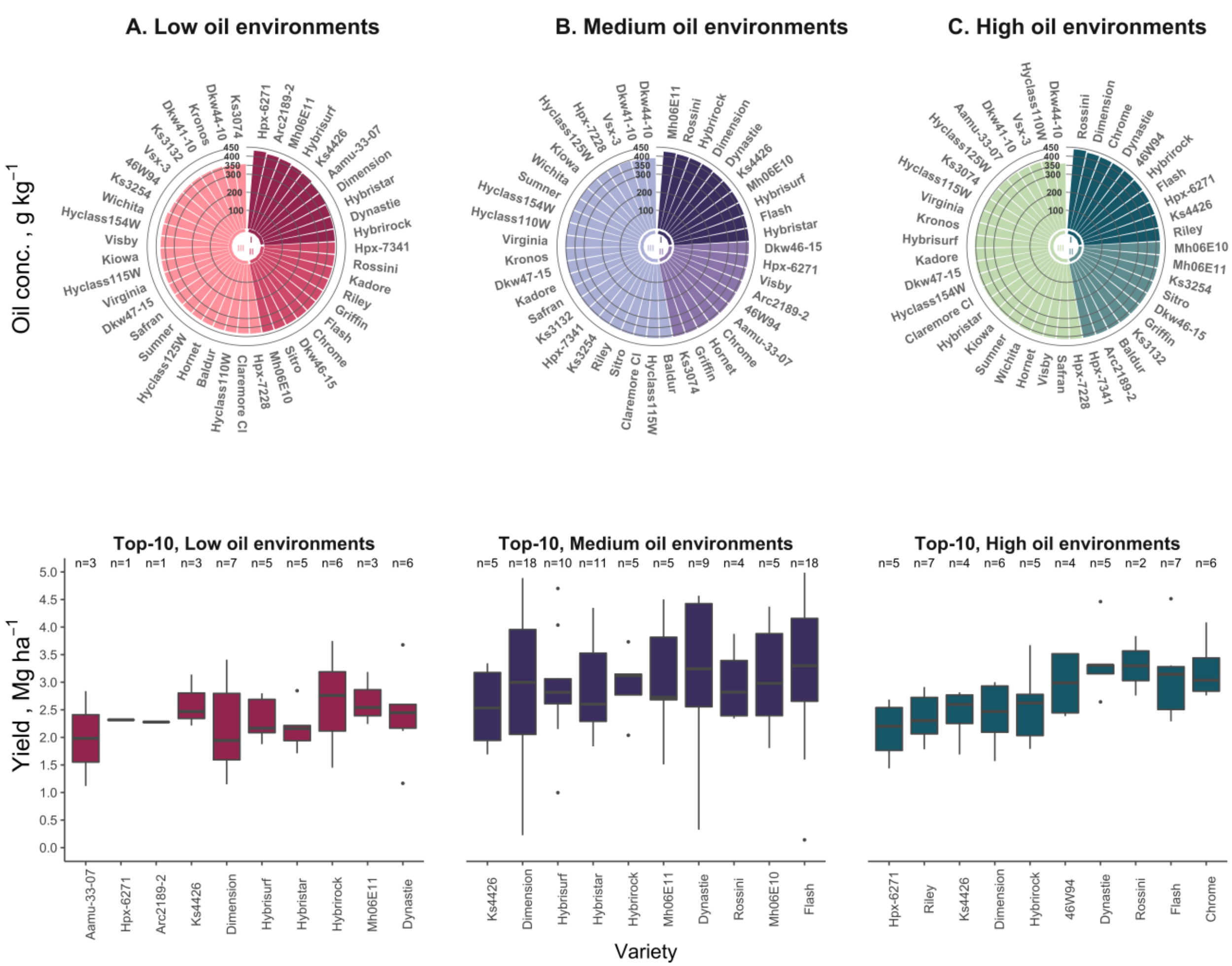

**Figure 3.4.** Genotype oil and yield performance comparison by expected oil environment (A: Low, B: Medium, C: High). All varieties (n = 43) were tested at more than 10 different site-years, and at least once at each oil environment (n = 1-34). Within each environment, seed oil concentrations values represent the average for each genotype, while classes I, II, and III (represented with different color shadings), depict the top-10, top-20, and the rest of varieties, respectively. Yield boxplots represent the interquartile range (25-75$^{th}$ percentiles) for yield, the horizontal line representing the median (50$^{th}$ percentile), whiskers range from 5$^{th}$ to 95$^{th}$ percentiles of yield distributions, and dots represent distribution outliers.

**Table 3.1.** Description of environmental variables during the seed filling period (means).

| Variable | Unit | Abbreviation | Calculation |
|---|---|---|---|
| Mean daily mean temperature | °C | meanTmean | (Tmax +Tmin)/2 |
| Cumulative precipitation | mm | cPrecip | - |
| Photothermal quotient | MJ $^{m-2}$ / °C.d. | PTQ | - |
| Shannon diversity index | 0-1 (uneven - even) | SDI | - |
| Cumulative growing degree days | °C.d. | cGDD | SUM (Tmax +Tmin)/2 |

**Table 3.2.** Summary statistics for meteorological variables [cumulative growing degree days (cGDD), cumulative precipitation (cPrecip_mm), mean daily temperature (meanTmean), photothermal quotient (PTQ), and Shannon diversity index (SDI)] during each phase of the seed filling period of all site-years. SD = Standard Deviation, CV = Coefficient of Variation, P1 = Phase 1, P2 = Phase 2, P3 = Phase 3.

| **Variable** | **Unit** | **Min** | **Mean** | **Max** | **SD** | **CV (%)** |
|---|---|---|---|---|---|---|
| cGDD_P1 | | 135 | 355 | 588 | 72 | 20.2 |
| cGDD_P2 | °C.d. | 159 | 362 | 591 | 71 | 19.7 |
| cGDD_P3 | | 152 | 373 | 616 | 75 | 20.0 |
| cPrecip_mm_P1 | | 0 | 82.3 | 235 | 53 | 64.6 |
| cPrecip_mm_P2 | mm | 0 | 74.8 | 269 | 57 | 75.8 |
| cPrecip_mm_P3 | | 0 | 61.7 | 170 | 46 | 74.9 |
| meanTmean_P1 | | 11.6 | 15.4 | 20.6 | 1.9 | 12.3 |
| meanTmean_P2 | °C | 13.9 | 19.0 | 25.9 | 2.2 | 11.6 |
| meanTmean_P3 | | 16.9 | 21.7 | 25.2 | 2.1 | 9.7 |
| PTQ_P1 | MJ $^{m-2}$ / | 1.0 | 1.4 | 2.2 | 0.3 | 21.4 |
| PTQ_P2 | °C.d. | 0.9 | 1.2 | 1.7 | 0.2 | 16.7 |
| PTQ_P3 | | 0.8 | 1.1 | 1.4 | 0.1 | 9.1 |
| SDI_P1 | 0-1 | 0 | 0.6 | 1.0 | 0.2 | 33.3 |
| SDI_P2 | (Uneven - | 0 | 0.5 | 0.9 | 0.2 | 40.0 |
| SDI_P3 | Even) | 0 | 0.5 | 0.8 | 0.2 | 40.0 |

# Chapter 4 - Effects of heat and drought on canola seed yield and nutritional quality: A meta-analysis

*Under review in Field Crop Research*

## Abstract

Canola (*Brassica napus* L.) is a major oilseed crop for edible and industrial uses. Climate change, influenced by extreme heat and drought conditions, can affect canola seed yield and quality (protein and oil) limiting production demands. However, estimating the influence of future climate in such complex traits is challenging due to biophysical interactions and crop adaptations to abiotic stresses. To better understand how drought and heat affects canola seed yield and quality, a meta-analysis was executed to compile a total of 37 papers gathering 1794 observations. The aims of this research were to i) quantify the impact of different timing and duration of heat and drought stresses, on canola seed quality (oil and protein concentration), seed yield, and oil yield, and ii) study the effect of short-term stresses on seed quality, yield, seed number, and seed weight. This research found that oil yield was reduced for heat (28%) and drought (20%), short stresses had greater impacts on seed yield and quality during pod setting than in other growth stages, and seed number and seed weight showed similar reductions during pod setting stresses. Results from this meta-analysis provide critical information for breeding programs and the future direction of canola production.

## 4.1 Introduction

Canola (*Brassica napus* L.) is a rapeseed crop with edible oil quality and potential for biofuel production gaining worldwide attention. Canola seeds contain high oil (40-45%) concentration, with the accumulation of triacylglycerol (TAG) as seed oil storage representing 30-to-60% of seed weight (Ohlrogge & Browse, 1995). In a simplified way, the oil quality is determined by the composition of fatty acids (FA), with some FA more desirable than others based on end uses. For edible oil, the nutritional value and stability of the oil depend on the distribution of unsaturated double bonds, while for industrial oil feedstock the FA carbon chain length is more relevant (Singer et al., 2016). Similarly, canola oil is used for biodiesel and its quality is highly related to the FA composition (Durrett et al., 2008). The use of biodiesel offers advantages such as providing a renewable source with less greenhouse gas emissions (Hill et al., 2006).

From a global perspective, there are three main types of canola cropping systems widely adapted for different growing environments (Kirkegaard et al., 2021). Winter canola, sown in the fall and harvested in summer (mostly in Europe, China, and United States regions); spring canola, sown in spring and harvested in summer (mostly in North America); and other spring canola, sown in the fall and harvested in spring (mostly in Australia, India, and South America). From 2018 to 2020 period, the major global producers of rapeseed were Canada, China, and India with roughly 60% of global production and harvested area. However, the highest seed yields are generally achieved by winter types in Germany, England, and France, with more than two-fold yield relative to the global average (FAOSTAT, 2022). These winter canola types are relevant in intermediate latitude regions in China, Germany, France, Poland, England, and in some smaller areas of the US, Australia, Canada, and Chile (Kirkegaard et al., 2021). Due to the increase frequency of extreme heat and drought events during summer months with climate change, increased adoption of winter canola crops is expected as a feasible option for diversifying agricultural systems in the future.

Canola seed yield and quality can be affected by temperature (Canvin, 1965; Faraji, 2012; Pokharel et al., 2021; Si et al., 2003) and water stresses (Champolivier & Merrien, 1996; Faraji et al., 2009; Farré et al., 2001; Jensen et al., 1996). In China, farmers only achieved between 37 to 56% of the yield potential (Zhang et al., 2020). In North America, the average canola yield in the 2000-2014 period was 50% lower than attainable yields (Assefa, Prasad, et al., 2018).For the US, seed oil concentration ranged from 30 to 50 % (Assefa et al., 2014) while in other regions such as Europe varied from 45 to 50% (Gunasekera et al., 2006). However, the magnitude of these effects across a broad range of environmental and management conditions for canola remains unclear. Because of the ongoing expansion of canola production into warmer and drier marginal areas, understanding the physiological impact of temperature and water stresses, and their interaction, is a major target and current critical research knowledge gap.

Refining our understanding of how environmental drivers affect seed yield and quality (oil and protein) will assist in providing new opportunities to expand canola production, increase crop diversification, and satisfy edible oil and biofuel demands. The aims of this research were to i) quantify the impact of different timing and duration of heat and drought stresses, on canola seed quality (oil and protein concentration), seed yield, and oil yield, and ii) study the effect of short-term stresses on seed quality (oil and protein), yield, and its main yield components (seed number and seed weight).

## 4.2 Materials and Methods

### Literature review

Published articles about canola were compiled using Web of Science ®, Science Direct ®, CAB Direct ®, and Google Scholar ® (limited to the first 100 most relevant articles) databases. The search was conducted between February and March 2022. The keywords on the search criteria were: (rapeseed OR canola) AND (oil) AND (yield) AND (heat OR drought OR water OR temperature) AND (content OR concentration). Only articles published in English language, agronomy, plant sciences, environmental sciences, and agriculture multidisciplinary research areas were selected.

A total of 873 article titles and abstracts were screened with a systematic literature review process using the *read_bibliography*, *find_duplicates, screen_titles, and screen_abstracts* functions included in the r*evtools* r-package (Westgate, 2019). The criteria for selecting articles were to focus only on articles comparing the effects of heat or drought on seed yield and/or quality (oil, protein). All analyses and systematic reviews were conducted within the R software program (R Core Team, 2022).

After the first screening (total 873 papers), 86 articles were selected for individual manual screenings, and later, all the data observations were pooled in the final database. Both controlled and field studies evaluating heat and drought were selected, disregarding studies evaluating environmental effects such as total rainfall or mean temperatures during the crop growing season. Studies in growth chambers, greenhouses, or rainfed shelters (only for drought), were grouped as a controlled environment, otherwise presented as field study conditions. When other agronomic factors were part of the treatment structure, data points were only included if factorial combinations were equally assigned and reported across treatments. Only studies of heat stress during the daytime were considered since only three out of nine papers reported nighttime temperature effects (representing a small sample size for a relevant analysis). A total of 29 studies were selected for the effect of drought stress and 8 for heat stress, with a total of 1794 observations (Table 4.1) from 12 countries and 37 locations (Figure 4.1).

***Pooled data and treatments classification***

Observations were pooled either from tables or using *Web Plot Digitizer* (Rohatgi, 2021) online tool. Within each selected study, specific criteria were followed to classify different treatments. Treatments were classified into the combination of two stress factors (Heat or Drought), two study experimental conditions (Controlled or Field), three stress durations (Control, Short, or Long), and three stress timings (Control, Early, or Late). For the duration of the stress, the data was classified as ‘short duration’ if the stress was imposed during one specific growth stage (or a short period of time) and later restored to normal conditions. Otherwise, if the stress was implemented in several growth stages (or for a long span), the data was classified as ‘long duration’. For the timing treatments, when the stress was imposed before the end of flowering, the data were classified as ‘early’. When the stress was imposed after the beginning of pod setting, the data were classified as ‘late’ stress. Data were classified as ‘control’ when no stress treatments were imposed. For drought studies, the most watered treatments were classified as the control.

Seed oil and protein values were expressed as percentages (%). Seed yield for controlled conditions was expressed or (when possible) converted to g plant$^{-1}$, while for field conditions to g m$^{-2}$. If seed moisture was reported, all seed yield values were adjusted to an 8.5% moisture level. When not reported, oil yield observations were calculated by multiplication of oil (%) and seed yield values. For studies with short stress duration, when yield components were reported, seed number (seeds plant$^{-1}$ or m$^{-2}$) and seed weight (1000 seeds) were added to the database to study the effect of stresses on these components. Within each study, data observations referred to the combination of year, treatment, and genotype.

## Analysis

A Bayesian random effects meta-analysis was conducted to estimate the response of variables to each type of stress and treatment combination. The effect sizes ($y_i$) used to compare the response of each treatment relative to the control, were derived using the natural log-transformed ratio between the mean of each response variable for the treatment and control value (Hedges et al., 1999; Lajeunesse, 2011) (Equation 4.1),

$$yi = lnRRi = ln\left\{\frac{\bar{x}\,Treatment}{\bar{x}\,Control}\right\}. \qquad \textbf{Equation 4.1}$$

Effect sizes of studies were weighted ($w$) inversely proportional to the variance, with variance ($v$) of each study ($i$) calculated as (Hedges et al., 1999):

$$vi = \frac{1}{wi} = \left(\frac{(SD_{Treatment})^2}{n_{Treatment} * X_{Treatment}}\right) + \left(\frac{(SD_{Control})^2}{n_{Control} * X_{Control}}\right), \qquad \textbf{Equation 4.2}$$

where $n$ indicates the data point numbers included in each of the groups, $X$ indicates the mean value of each group, $SD_{Treatment}$ and $SD_{Control}$ were the obtained standard deviations for each of the groups that were reported or could be derived from other quantities such as standard error, least significance difference (LSD), or coefficient of variation. When variance information was not reported, we developed a hierarchical framework within the Bayesian framework to estimate the respective SDs while accounting for the uncertainty due to the SDs missingness (Varela, 2015). Assuming variances follow a positive gamma distribution, a gamma distribution was first estimated for each variable and treatment group using maximum likelihood estimation on the available SDs data with the *gamlss* package (Rigby & Stasinopoulos, 2005). The estimated gamma distribution was used as a prior in the Bayesian model for estimating the missing SDs and calculating $vi$. Only for the subset analysis on yield components (seed number and seed weight), for which no data was available to estimate prior distributions for SDs, we used an alternative weighting based on the number of replicates (Hedges & Olkin, 1985) (Equation 4.3),

$$\boldsymbol{vi} = \frac{\mathbf{1}}{\boldsymbol{wi}} = \left(\frac{\boldsymbol{n_{Treatment}} + \boldsymbol{n_{Control}}}{\boldsymbol{n_{Treatment}} \cdot \boldsymbol{n_{Control}}}\right). \qquad \textbf{Equation 4.3}$$

The overall meta-analytic model was defined as:

$$\boldsymbol{yi|\theta i \sim N(\theta i, vi)}, \qquad \textbf{Equation 4.4}$$

$$\theta i \sim N(\mu, tau^2),$$

where for a set of independent studies $i$=1,…., $k$ , $yi$ is the observed effect size, assumed to be unbiased towards $\theta i$ and normally distributed with within-study variances ($vi$), and $\theta i$ is normally distributed with $\mu$ denoting the mean true effect and $tau^2$ the between-study variance. Weakly informative prior distributions were used for $\mu$ ($\mu \sim N(0{,}1000)$) and $tau^2$ ($tau^2 \sim Unif(0{,}10)$). To compare the effect of timing, duration, and stage of stress imposed, a moderator analysis was conducted by adding a ‘fixed’ effect factor ($\alpha i$) to the model,

$$\boldsymbol{yi|\theta i, \alpha i \sim N(\theta i + \alpha i, vi)}, \qquad \textbf{Equation 4.5}$$

with a weakly informative prior distribution $\alpha i \sim N(0{,}1000)$. Heterogeneity between studies was assessed via the $I^2$ statistic to quantify the percentage of total variation across studies that can be attributed to heterogeneity rather than an experimental error (Higgins et al., 2003).

All inferences were based on posterior samples obtained via Markov Chain Monte Carlo (MCMC) simulation using the *rjags* package in R (Plummer, 2015). The MCMC algorithm was run in three chains with 10,000 adapt/burn-in iterations discarded and further 10,000 model iterations for inference. Convergence was assessed by Gelman-Rubin diagnostics and visual inspection of density and trace plots (Gelman & Rubin, 1992). After model fitting, for better interpretation, effect size units were backtransformed and reported as a percentage of the treatment group response over the control using the following equation:

$$\boldsymbol{Effect}\ (\%) = \left\{\frac{\bar{x}\,\boldsymbol{Treatment}}{\bar{x}\,\boldsymbol{Control}} - \mathbf{1}\right\} * \mathbf{100}\ .$$ **Equation 4.6**

For visualization of treatment comparison within each variable and stress, forest plots are presented using the *forest* function with the *metaphor R* package (Viechtbauer, 2010).

## 4.3 Results

***Seed oil concentration and protein concentration summary***

For each seed quality response variable and treatment group combination, the number of observations, minimum, mean, maximum, and standard deviation values were calculated and summarized in Table 4.2.

***Overall responses of seed quality and yield to heat and drought stresses***

For seed oil concentration, heat stress (Figure 4.2-A) showed an overall reduction of 5%. A large reduction of 14% was reflected when stress was imposed under controlled conditions at early timing (before the end of flowering) for a long duration (in several growth stages) and a reduction of 5% with the same timing and duration but under field conditions. In addition, significant reductions of 5% were reflected under controlled conditions at late timing (after the beginning of pod setting) for a short period (one specific growth stage). Overall, drought stress (Figure 4.2-B) did not affect seed oil concentration. However, was significantly affected specifically under field conditions, with a 3% reduction at early timing for a long duration, and a 2% increase at late timing for short duration stress.

For seed protein concentration, heat stress under controlled conditions (Figure 4.2-C) showed an overall increment of 5%. Specifically, the stress imposed for a short duration increased protein concentrations by 12% at early timing and 5% at late timing, while on the other hand, at early timing for a long duration, caused a reduction of 14%. Drought stress (Figure 4.2-

D) did not affect seed protein concentration overall. Only under field conditions, stress at late timing for a short period, significantly reduced protein concentration levels by 7%.

For seed yield, heat stress under controlled conditions had an overall negative impact of 21%. Stress at early timing caused reductions of 39% for short and 20% for long durations. Late timing stress for a short duration also reduced seed yield by 21% (Figure 4.2-E). Overall, drought stress negatively impacted yield by 21%, with a large reduction of 39% when stress was imposed under controlled conditions at early timing for a long period and 9% when the stress was under field conditions at late timing for a short period (Fig. Figure 4.2-F).

For oil yield, heat stress under controlled conditions (Figure 4.2-G) showed an overall reduction of 28%, with the largest reduction of 66% when stress was imposed at early timing for a short duration and the lowest of 24% when imposed at late timing for a short duration. Drought stress (Figure 4.2-H) showed an overall reduction of 20%, with a large reduction of 42% when stress was imposed at late timing for a short period under controlled conditions, while under field conditions, the same timing and duration reduced oil yield by 9%.

***Overall responses to short heat and drought stress***

Overall effects sizes of short heat and drought combined stresses were calculated to differentiate timing effects on each response variable (Figure 4.3). For seed oil concentration, stresses during pod setting resulted in the largest reduction by 6%, with neutral effects on vegetative and flowering and slightly positive for seed filling (Figure 4.3). Seed protein concentration increased by 14% in response to stress during flowering, and slightly less when the stress was imposed during pod set. Lastly, for seed yield and oil yield, short stresses resulted in the largest impact when imposed during pod setting with reductions of 36 and 54%, respectively (Figure 4.3). For these two traits, short stresses always resulted in negative impacts regardless of the timing, but the seed yield was the main driver on the changes for oil yield for the vegetative and flowering stress timings.

***Effects of combined stress on yield components, seed number and seed weight***

Overall effects sizes of short heat and drought stresses were quantified for specific crop growth stages on seed number and seed weight (Figure 4.4), using a subset of the main database (8 out of 37 papers). Although there was a large range of variation for each response variable at each crop growth stage, both had the highest impact under short stress during pod setting with reductions of 24% for seed number and 26% for seed weight (Figure 4.4).

In summary, oil yield was significantly reduced for heat (28%) and drought (20%) stresses. Seed oil concentration and seed yield reductions had greater reductions with short stresses during pod setting than in other crop growth stages (Figure 4.3). Yield components (seed number and seed weight) were similarly reduced with short stresses during pod setting (Figure 4.4). With short stresses during flowering, seed oil concentration was not reduced, and final oil yield reduction was similar to the effect of stresses during both vegetative and seed filling. Effects of short stresses during flowering on yield components showed a trend of greater reductions in seed number than seed weight, with regulation of the seed weight only when early stresses conditions are reversed.

## 4.4 Discussion

This meta-analysis quantified the magnitude and direction of responses to heat and drought stresses of canola seed productivity and quality. Findings demonstrated that heat stress reduces seed oil concentration, yield, and oil yield while increasing seed protein concentration. Drought stress has neutral effects on seed oil and protein concentration but reduces final canola seed and oil yield. In a context of future canola production affected by climate change (Ray et al., 2019), this study provides critical knowledge to quantify the potential impact of heat and drought events on seed yield and seed quality (i.e., oil as the most relevant component).

The period between 14-35 days after flowering is a critical phase for determining the synthesis and storage of seed components (Appelqvist, 1982; Deng & Scarth, 1998; Fowler & Downey, 1970). During the seed filling period, seed oil accumulation is regulated by the photosynthetic carbon assimilation (Bennett et al., 2011; Hua et al., 2012). High temperatures affect the enzymatic panel involved in the oil biosynthesis pathways, reducing the available photo-assimilates for TAG biosynthesis and seed oil accumulation, with larger impact for during late- relative to early- seed filling phases, affecting the final FA composition (Baud & Lepiniec, 2010). Contrasting results were reported in the scientific literature for heat and drought effects in oil concentration. Heat stress had negative (Faraji, 2012; Singer et al., 2016; Zhang et al., 2014; Zhu et al., 2012) or positive effects (Pokharel et al., 2021), while drought had negative (Jensen et al., 1996; Champolivier and Merrien, 1996; Faraji et al., 2009; Aslam et al., 2009) or neutral effects (Elferjani & Soolanayakanahally, 2018; Zarei et al., 2010). However, the magnitude of the effects on seed oil concentration was largely affected by the genotype-by-environment (G x E) interaction across studies (Pritchard et al., 2000; Sinaki, 2009; Zhang et al., 2014).

The indeterminate growth habit of canola can help to understand some of these contrasting results on the impact of abiotic stresses, with the timing and duration of the stress playing a major role. McGregor (1981) introduced the "recovery" term in rapeseed, explaining that undesirable stress effects can be compensated by producing additional branches, flowers, inflorescences, or more seeds per pod after stress is released. Pokharel et al. (2021) found seed oil concentration increments when plants were exposed to a short 14-days heat stress during flowering, suggesting a post-stress recovery and phenotypic plasticity. Our results indicate that oil concentration is reduced by 5% with heat stresses, with larger reductions when imposed early and for a long period. Drought stresses had neutral effects, however, specific stresses reduced (early-long) or increased (late-short) seed oil concentration. Furthermore, under heat stress, our results evidenced the critical trade-off between seed oil and protein concentration reported for canola (Aksouh-Harradj et al., 2006; Rondanini et al., 2014), highlighting the importance of assessing seed quality from an integrated approach for both seed oil and protein components.

From a productivity standpoint, current research showed greater seed yield sensitivity when heat stress was imposed at flowering. Similarly, Angadi et al. (2000) reported that a short heat stress of 35/15°C (day/night) had higher seed yield reductions when imposed during flowering (52%) than during pod setting (18%). Gan et al. (2004) observed 78% and 96% yield reductions with 28/18°C and 35/18°C short heat stresses during flowering. Pollen viability, germination, and tube growth can be reduced when exposed to temperatures below or above the optimum (23.6 °C), resulting in lower germination rates, micropyle penetration, fertilization, and post-fertilization events (Singh et al., 2008; L. Young et al., 2004). Flower abortion rates, malformations of reproductive organs (Angadi et al., 2000; Polowick & Sawhney, 1988), prematurely end of flowering limiting seed set (Faraji et al., 2009), number of opened flowers, and pod/flowers ratio will determine yield potential (Morrison & Stewart, 2002). Pod-filling stages are also sensitive to heat stress (Weymann et al., 2015), high temperatures during seed filling, will shorten the duration and potentially reduce seed yield (P. J. Hocking et al., 1997). Our review study confirms that early short heat had a greater impact on seed yield (39%) than early long heat (20%) stress across a wide range of canola growing conditions, plausible via an impact on both seed number and weight (Figure 4.4).

Under drought, water limitations during seed set can decrease seed yield, while later during the seed filling period can reduce seed size (Andriani et al., 1991; Snyder et al., 1982) affecting final seed oil concentration. Seed yield is closely linked to the amount of water available between flowering and mid-pod development (Berry & Spink, 2006; Mendham et al., 1981) which coincides with the critical period described by Kirkegaard et al. (2018). Studies reported that drought stress caused seed yield reductions at flowering (Rahnema & Bakhshandeh, 2006) and during late flowering to maturity (Pasban et al. 2000). Our results indicate that all drought stress types reduced seed yield, and only stress imposed late-long under controlled conditions had neutral effects.

Selecting tolerant genotypes is considered one of the best strategies to reduce negative abiotic stress effects. In some environments, genotypes with early relative maturity will also help mitigate heat and drought stresses, ending the flowering period before stresses occur. Future research should focus on developing new strategies involving direct regulation of multiple genes that could resist a wide range of environmental stresses. In addition, exploring the best management practices to avoid stress under critical periods must be considered to mitigate the negative impacts on oil yield and quality from future climate scenarios. This meta-analysis summarizes data from different studies, dissecting the overall impact of abiotic stress timing and duration for seed yield and quality. Identifying the magnitude and direction of canola seed productivity and quality responses to heat and drought stresses can be used in future research to mitigate unfavorable future climate scenarios.

## 4.5 Conclusions

Projected climate scenarios, with extreme heat and drought conditions, could challenge global food and energy security. This research reported that (i) early heat stress (before end of flowering) had the highest impact on canola seed oil concentration when imposed for a long duration (several growth stages), and on protein concentration, seed yield, and oil yield when imposed for a short duration (one growth stage); (ii) drought stress caused the highest reduction on seed yield at early timing for a long duration, while on oil yield after beginning of pod setting for a short duration; (iii) comparisons within short stresses had greater reductions on seed oil concentration, yield, and oil yield during pod setting than in other crop growth stages; and (ii) short stresses during pod setting showed similar negative effects in both seed number and seed weight, with larger reduction in seed number with a stress during flowering. Improving knowledge of the effect of abiotic stressors provides critical information for breeding programs and the future direction of canola production.

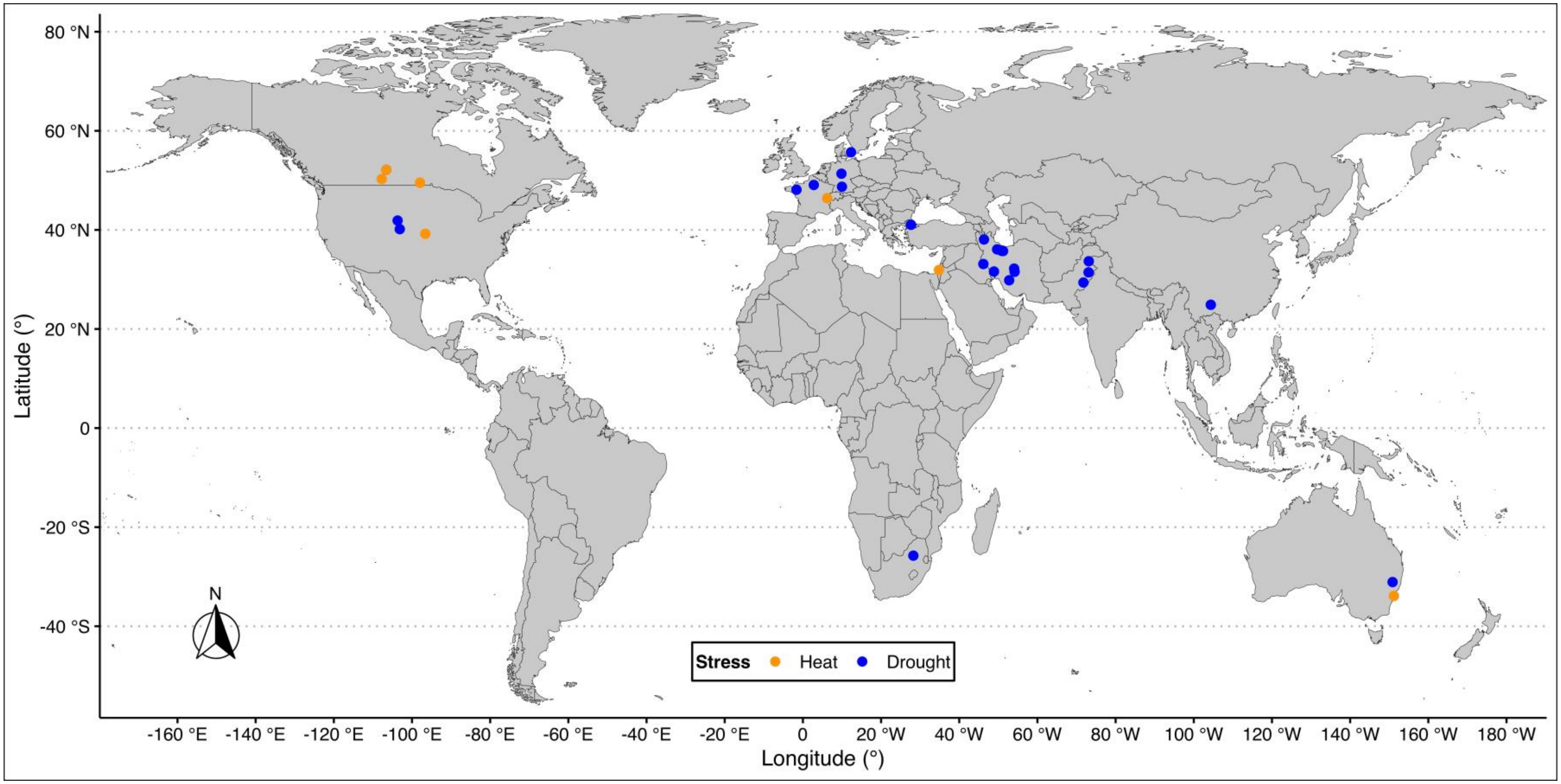


**Figure 4.1.** Global map (latitude and longitude) of selected canola crop studies distributed in different continents, representing a total of 12 countries and 37 locations. For each location, different colors refer to the study of different stress factors, heat stress (in orange) and drought stress (in blue).

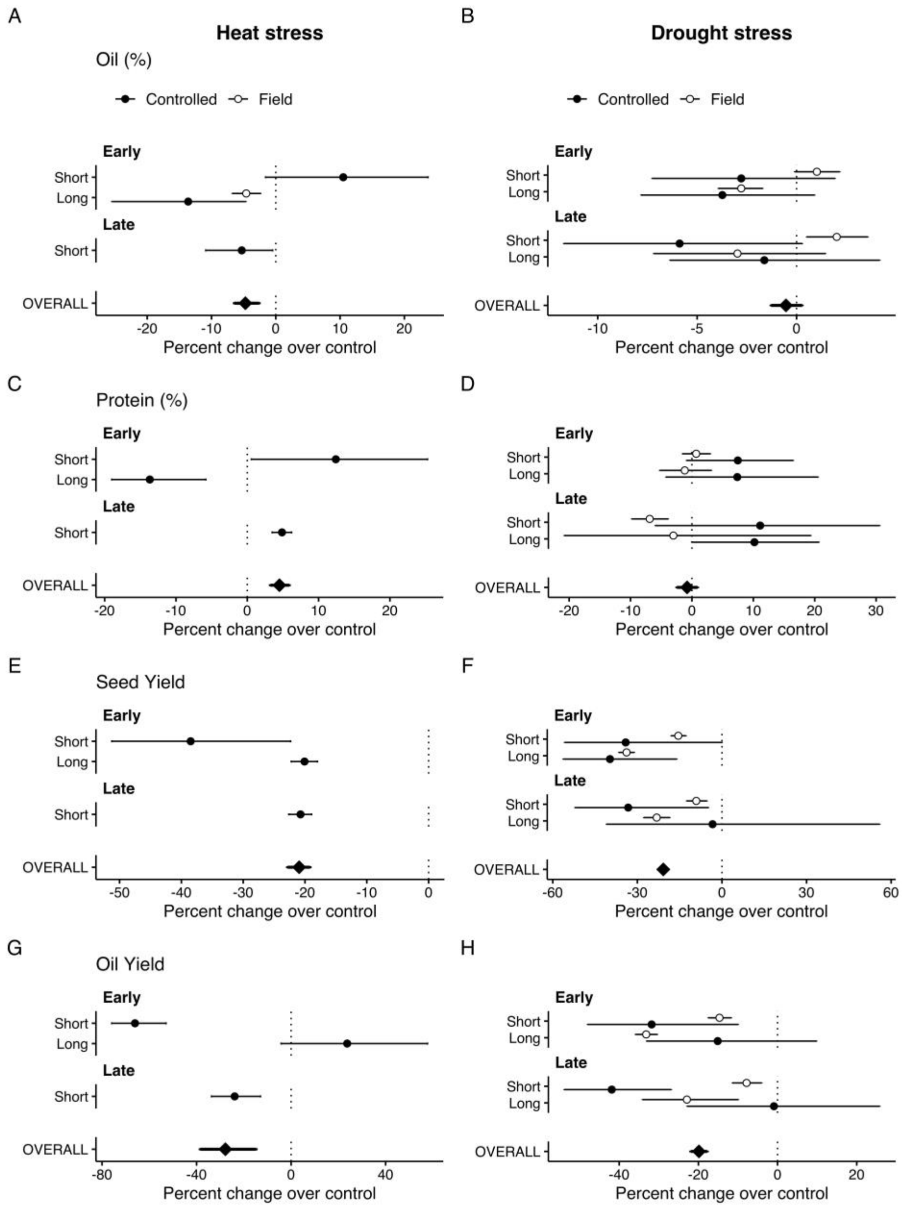


**Figure 4.2.** Forest plot summarizing effect sizes of treatment combinations (different stresses) under two experimental conditions with four response variables. Experimental conditions are represented with black circles (controlled), and white circles (field). Heat (Panel A, C, E, and G), drought (Panel B, D, F, and H), timing (Early and Late), duration (Short and Long), and overall (black triangles) stress effects sizes (%) on seed oil and protein concentration, seed yield, and oil yield. Early timing stress is imposed before the end of flowering, and late timing after the beginning of pod setting. Short duration stress is imposed during one specific growth stage, long duration stress is implemented in several growth stages. Effect sizes and 95% confidence intervals (CI) are expressed as % of response against the control treatment. Circle symbols represent the median point estimates and whiskers depict their respective 95% CI. The weight of each treatment is expressed as the line thickness and symbol size as a percentage of the overall model. The vertical dotted line represents 0 % of treatment response over the control.

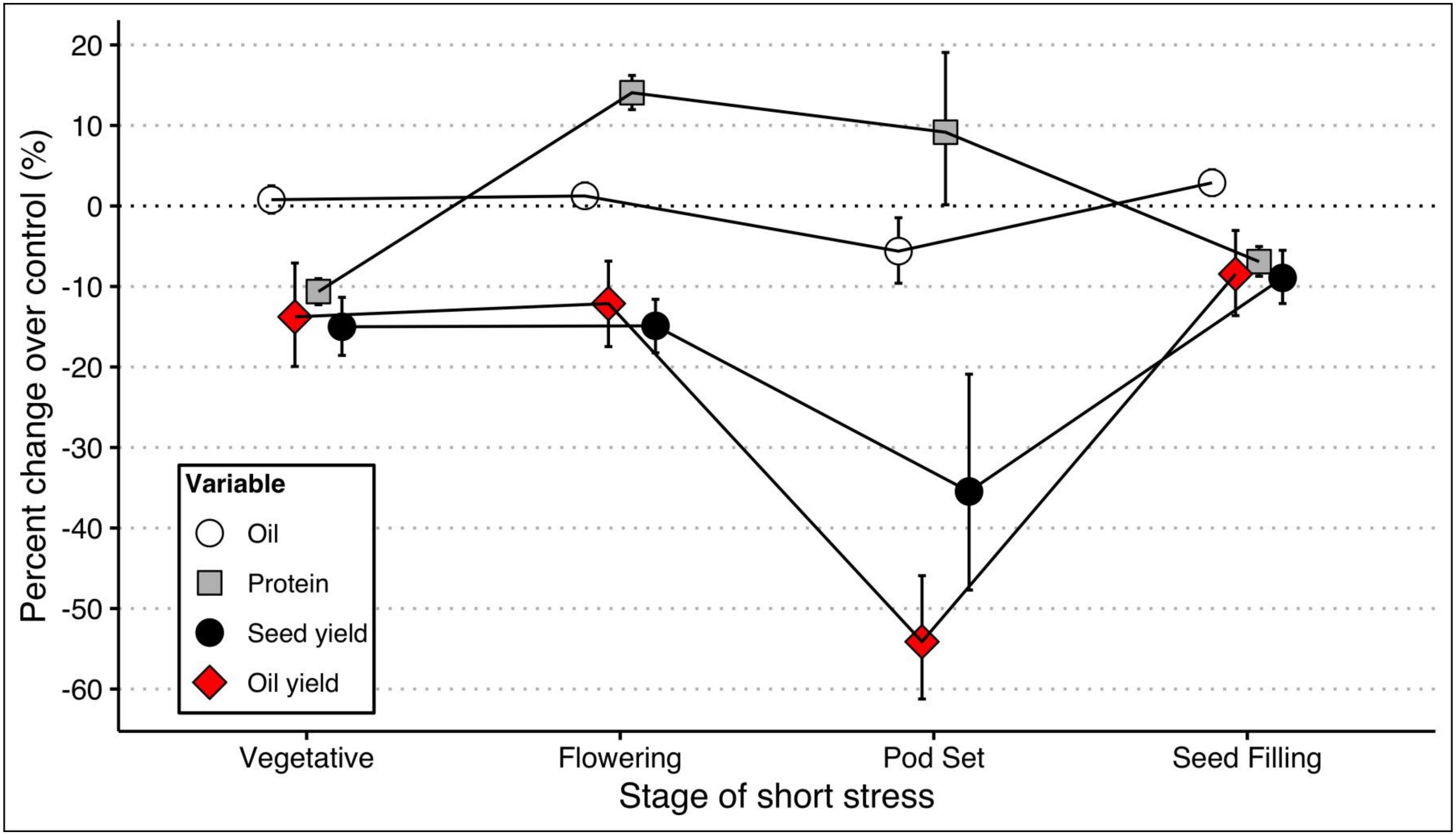


**Figure 4.3.** Overall effects sizes of short stresses for seed oil (White circles) and protein (Grey squares) concentration, Seed Yield (Black circles), and Oil Yield (Red triangles). Effect sizes and 95% confidence intervals (CI) are expressed as % of response against the control treatment. Symbols represent the median point estimates and whiskers depict their respective 95% CI.

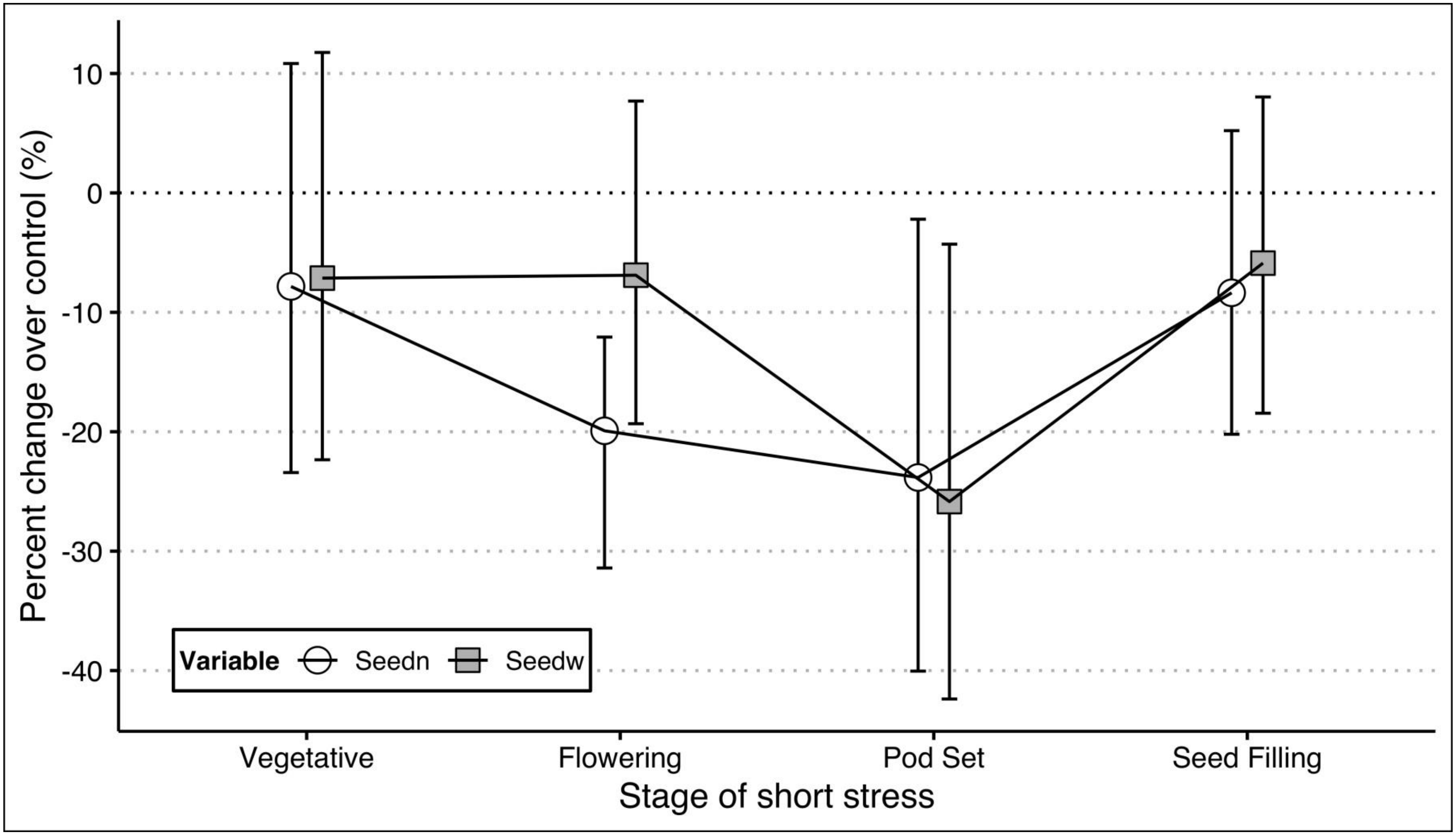


**Figure 4.4.** Overall effects sizes of short stresses for seed number (Seedn, white circles) and seed weight (Seedw, grey squares). Effect sizes and 95% confidence intervals (CI) are expressed as % of response against the control treatment. Symbols represent the median point estimates and whiskers depict their respective 95% CI.

**Table 4.1** Summary and description of pooled studies. Authors, environment (city, country), latitude and longitude coordinates, stress factor (heat and drought), condition (controlled and field), and number of genotypes of studies for stress effects in seed oil, protein, and yield. The term “Mean” in the column termed as Genotypes, describes those authors reported the overall average of genotypes.

| Authors | Environment | Latitude | Longitude | Factor | Condition | Genotypes |
|---|---|---|---|---|---|---|
| Abbasian and Shirani Rad, 2011 | Karaj, Iran | 35.80 | 50.98 | Drought | Field | 4 |
| Ahmadi and Bahrani, 2009 | Shiraz, Iran | 29.83 | 52.77 | Drought | Field | Mean |
| Aksouh et al., 2001 | Sidney, Australia | -33.89 | 151.19 | Heat | Controlled | 3 |
| Aksouh et al., 2006 | Wagga Wagga, Australia | -33.89 | 151.19 | Heat | Controlled | 3 |
| Angadi et al., 2000 | Swift Current, Canada | 50.28 | -107.76 | Heat | Controlled | 3 |
| Bilibio et al., 2011 | Witzenhausen, Germany | 51.34 | 9.86 | Drought | Controlled | Mean |
| Bouchereau et al., 1996 | Rennes, France | 48.11 | -1.64 | Drought | Controlled | 3 |
| Champolivier and Merrien, 1996 | St Pathus, France | 49.07 | 2.79 | Drought | Controlled | Mean |
| Danesh-Shahraki et al., 2008 | Ahvaz, Iran | 31.60 | 48.88 | Drought | Field | Mean |
| Din et al., 2011 | Islamabad, Pakistan | 33.68 | 73.13 | Drought | Controlled | 5 |
| Drebenstedt et al., 2020 | Stuttgart, Germany | 48.72 | 9.98 | Drought | Field | Mean |
| Elferjani and Soolanayakanahally, 2018 | Saskatoon, Canada | 52.15 | -106.58 | Drought | Controlled | Mean |
| Elferjani and Soolanayakanahally, 2018 | Saskatoon, Canada | 52.15 | -106.58 | Heat | Controlled | Mean |
| Eyni-Nargeseh et al., 2020 | Karaj, Iran | 35.75 | 50.91 | Drought | Field | 17 |
| Feizabadi et al., 2021 | Karaj, Iran | 35.81 | 51.03 | Drought | Field | 6 |
| Gültaş and Ahi, 2020 | Tekirdaƒü, Turkey | 41.03 | 27.65 | Drought | Field | Mean |
| Gauthier et al., 2017 | Changins, Switzerland | 46.40 | 6.23 | Heat | Controlled | 3 |
| Ghobadi et al., 2006 | Mollasani, Iran | 31.60 | 48.88 | Drought | Controlled | Mean |
| Ghobadi et al., 2006 | Mollasani, Iran | 31.60 | 48.88 | Drought | Field | 3 |
| Majnooni-Heris et al., 2014 | Karkaj, Iran | 38.06 | 46.33 | Drought | Field | Mean |
| Huang et al., 2015 | Luoping, China | 24.88 | 104.35 | Drought | Field | 37 |
| Jabbari et al., 2018 | Yazd, Iran | 31.55 | 54.16 | Drought | Field | 3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Jensen et al., 1996 | Copenhague, Denmark | 55.67 | 12.30 | Drought | Controlled | Mean |
| Keshavarz, 2020 | Tehran, Iran | 35.74 | 51.21 | Drought | Field | Mean |
| Khodabin et al., 2021 | Karaj, Iran | 35.80 | 50.98 | Drought | Field | Mean |
| Koscielny et al., 2018 | Manitoba, Canada | 49.52 | -97.95 | Heat | Field | 25 |
| Mirzaei et al., 2013 | Mehran, Iran | 33.12 | 46.17 | Drought | Field | 4 |
| Moaveni et al., 2010 | Karaj, Iran | 35.81 | 51.03 | Drought | Field | 3 |
| Nielsen, 1997 | Akron, USA | 40.15 | -103.14 | Drought | Field | Mean |
| Pavlista et al., 2016 | Nebraska, USA | 41.89 | -103.68 | Drought | Field | Mean |
| Pokharel et al., 2021 | Manhattan, USA | 39.21 | -96.59 | Heat | Controlled | 6 |
| Pokharel et al., 2021 | Manhattan, USA | 39.21 | -96.59 | Heat | Field | 5 |
| Raza et al., 2015 | Bahawalpur, Pakistan | 29.38 | 71.76 | Drought | Field | Mean |
| Shirani Rad and Abbasian, 2011 | Karaj, Iran | 35.81 | 51.03 | Drought | Field | 23 |
| Shirani Rad and Zandi, 2012 | Karaj, Iran | 35.75 | 50.91 | Drought | Field | 20 |
| Shirani Rad, 2012 | Karaj, Iran | 35.75 | 50.91 | Drought | Field | 34 |
| Shirani Rad et al., 2014 | Takestan, Iran | 36.07 | 49.70 | Drought | Field | 3 |
| Tahir et al., 2007 | Faisalabad, Pakistan | 31.43 | 73.08 | Drought | Field | Mean |
| Tesfamariam et al., 2010 | Pretoria, South Africa | -25.75 | 28.25 | Drought | Field | Mean |
| Wright et al., 1995 | Tamworth, Australia | -31.08 | 150.86 | Drought | Field | Mean |
| Yaniv et al., 1995 | Rehovot, Israel | 31.90 | 34.80 | Heat | Controlled | 2 |
| Zarei et al., 2010 | Maybod, Iran | 32.19 | 54.04 | Drought | Field | 3 |

**Table 4.2.** Summary statistics of seed oil concentration (%) and protein concentration (%). Number of observations (_Nobs), minimum (_Min), mean (_Mean), maximum (_Max), and standard deviation (_SD) for each response variable and treatment group. Treatments groups are a combination of stress factors (Drought or Heat), experimental conditions (Controlled or Field), timing (Early or Late), and duration (Short or Long). Early timing stress is imposed before the end of flowering, and late timing after the beginning of pod setting. Short duration stress is imposed during one specific growth stage, long duration stress is implemented in several growth stages.

| **Factor** | **Drought** | | | | | | | | | | **Heat** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Condition** | **Controlled** | | | | | **Field** | | | | | **Controlled** | | | | **Field** | |
| **Timing** | **Control** | **Early** | **Early** | **Late** | **Late** | **Control** | **Early** | **Early** | **Late** | **Late** | **Control** | **Early** | **Early** | **Late** | **Control** | **Early** |
| **Duration** | | **Long** | **Short** | **Long** | **Short** | | **Long** | **Short** | **Long** | **Short** | | **Long** | **Short** | **Short** | | **Long** |
| **Oil_Nobs** | 16 | 10 | 15 | 8 | 10 | 240 | 195 | 38 | 62 | 32 | 21 | 6 | 12 | 18 | 30 | 30 |
| **Oil_Min (%)** | 36.1 | 35.3 | 36.1 | 38.1 | 41.2 | 31.4 | 28.7 | 34 | 36.9 | 32.3 | 34.3 | 17.2 | 38.9 | 23.5 | 37.3 | 33.6 |
| **Oil_mean (%)** | 42.1 | 40.6 | 40.8 | 42.6 | 42.1 | 44.1 | 43.6 | 38 | 43.6 | 39.2 | 40.3 | 36.6 | 43.1 | 33.6 | 45.8 | 44 |
| **Oil_max (%)** | 46.5 | 46.2 | 45.5 | 46.5 | 42.8 | 54.7 | 64.1 | 41.6 | 64 | 56.8 | 45.6 | 42.4 | 46.3 | 42.3 | 48.3 | 47 |
| **Oil_SD** | 3.7 | 3.9 | 3.1 | 2.8 | 0.6 | 4 | 4.1 | 1.5 | 6.1 | 4.1 | 3.4 | 9.7 | 2.9 | 5.2 | 3.2 | 3.8 |
| **Protein_Nobs** | 16 | 10 | 15 | 8 | 10 | 240 | 195 | 38 | 62 | 32 | 21 | | 12 | 18 | 30 | 30 |
| **Protein_Min (%)** | 20.2 | 20.9 | 22.5 | 20.7 | 24.3 | 13.3 | 15.4 | 19 | 12.8 | 21 | 22.1 | | 19.8 | 24.9 | 23.9 | 25.3 |
| **Protein_Mean (%)** | 22.7 | 24.9 | 24.1 | 23.6 | 25.5 | 21.6 | 20.7 | 23.6 | 13 | 21.6 | 25.2 | | 21.7 | 27.9 | 25.1 | 26.7 |
| **Protein_Max (%)** | 30 | 32.8 | 25.6 | 25.4 | 26.7 | 24 | 24.2 | 29 | 13.4 | 22 | 27.4 | | 24.7 | 29.4 | 26.1 | 30.3 |
| **Protein_SD** | 2.9 | 3.7 | 1.2 | 1.9 | 1.7 | 2.4 | 2 | 3.6 | 0.3 | 0.5 | 1.6 | | 1.8 | 1.6 | 0.6 | 1.2 |

# Chapter 5 - Future climate change portrays mostly favorable impacts on winter canola yields in the United States


## Abstract

Climate change projections are expected to have great impacts on crop yields; however, the magnitude and direction of those impacts vary depending on specific regions and crop type (winter vs. summer). Future climate projection models forecast an increase in precipitation during winter and spring months, and a higher frequency of extreme drought and heat events during summer for most of the United States (US) territory. In this context, the adoption of winter crops could be a feasible option to mitigate the negative impacts on food and energy future supply, driven by climate change. Winter canola (*Brassica napus* L.) has the potential to adjust to these future weather conditions and to provide edible oil and biofuel feedstock to satisfy the forecasted demand increases. The aims of this study were to i) quantify changes in seed yield due to future climate scenarios, and ii) investigate the geographical changes in overall production over time and identify US regions suitable for future expansion of winter canola production. This study presented the first-time changes in winter canola yield over time (1997–2099), identifying that future weather projections (SSP-2.6 and SSP-8.5) are mostly favorable for winter canola US production, except for a few regions. Yield gains were found in the Great Plains and eastern US regions, ranging from 12 to 254 kg $ha^{-1}$. Although changes in future atmospheric CO2 concentrations are not accounted for, this crop modeling exercise permitted identifying potential changes in yield driven by climate change (mainly temperature and precipitation). Highest positive yield trends (11 kg $ha^{-1}$ $year^{-1}$) were observed during the end of the century (2080–2099) under the most extreme future climate conditions (SSP-8.5), maximizing yield gains (89 kg $ha^{-1}$). This research identifies the geographical areas for winter canola crop expansion, that can lead to satisfying future food and energy demands.

## 5.1 Introduction

Canola (*Brassica napus* L.) is an important rapeseed crop that provides high monounsaturated fatty acid oil for human consumption, feedstock for biofuel, and high protein meal for livestock consumption. This oilseed crop provides opportunities for diversification aiming for sustainability and securing long-term food production (Renard & Tilman, 2019). Inclusion of winter canola as an alternative to winter wheat (*Triticum aestivum* L.) monocropping has been proven to break weed and pest cycles (Angus et al., 2015; Kirkegaard et al., 2016). However, the prospective for winter canola expansion in the United States (US) is highly linked to winter survival, especially in production environments above latitude 35°N (Secchi et al., 2021).

In this context, canola hectares planted in the US have grown from 63 to 871 thousand hectares during the last 30 years (USDA-ERS, 2021). Furthermore, in the US, two canola types prevail in the production systems i) spring (sown in early spring and harvested in summer); and ii) winter (sown in the fall and harvested in summer) (Kirkegaard et al., 2021). Most canola production in the US is spring types, developed in Canada and adapted and grown in the Northern Plains and Pacific Northwest regions (USDA-ERS, 2021). In contrast, less hectares are planted of winter canola (mainly in warmer regions such as the Southern Plains), despite having greater yield potential (Christy et al., 2019; Page et al., 2021). Notwithstanding the challenges of winter survival, winter canola can achieve high yields with stand reductions of 50% (OMAFRA, 2011), and the correct selection of genotype and management practices for specific US environments can minimize winterkill (Assefa et al., 2014; Secchi et al., 2021). This last point becomes more relevant as the canola demand in the US increases (Ates & Bukowski, 2022), reflecting the need to identify regions where high canola potential yields can be achieved.

Future climate change projections are expected to have both positive and negative impacts on crop yields (Hasegawa et al., 2022; Ray et al., 2019). For the Northern US Plains, an increased frequency of extreme heat and drought events is forecasted, jeopardizing summer crop production including spring canola (Hasegawa et al., 2022; Qian et al., 2018). On the other hand, the projected increase of precipitation during winter and spring points out the relevance of exploring winter crops, such as winter canola, as alternatives to cope with future weather in central and eastern North America (Almazroui et al., 2021). Nevertheless, the direction of future climate impacts on crop yields will depend on the environment considered, the specific crop, and the definition of potential adaptation strategies (Zabel et al., 2021). Crop simulation models, once they are well parameterized with field data, are a useful tool to explore the impact of contrasting weather scenarios for different crops (Asseng et al., 2013; Porter et al., 2019; White et al., 2011).

Therefore, this study compiles a multi-environment dataset, containing more than two hundred winter canola site-years, with future climate scenarios using APSIM Next Generation (Holzworth et al., 2018) crop growth model to address yield variability across the US. The objectives of this study were to i) quantify changes in seed yield due to future climate scenarios, and ii) investigate the geographical changes in overall production over time and identify US regions suitable for future expansion of winter canola production.

## 5.2 Materials and Methods

### Observed dataset and response variables

The field observed dataset and management practices were curated from the National Winter Canola Variety Trial (NWCVT) (Stamm et al., 2019). The purpose of this national network of trials is to evaluate yield performance, winter survival, and crop phenology across many environments for different US states. The experimental design for each trial defined by site and year was a randomized complete block with three or four replications, depending on collaborator specifications. The entire dataset comprises 109 sites covering 34 US states for the 2003 – 2019 period and includes a total of 514 varieties. Only sites with seed yield, flowering date, and maturity date information were included in this study (75 sites, 260 site-years). For each site-year, management parameters retrieved from the dataset were planting date, nitrogen (N) fertilization, irrigation amount at planting and spring, irrigation date in the spring, plant density, and row spacing. Plant density was adjusted by winter survival and stand establishment rates. Due to missing information on initial N soil availability and applied N fertilization (in more than 50% of the site-years), this input was estimated following Kansas State University fertilization recommendations (Baltensperger et al., 2006; KSU, 2018) based on observed yield as a proxy of total crop N demand. A summary of the management practices per site is described in Table B1.

### Analysis

All simulations were performed using the Agricultural Production Systems sIMulator (APSIM) Next Generation software platform. APSIM is a modular modeling system that has been used in many applications, including farming systems design and assessment of climate forecasting (Holzworth et al., 2018). The APSIM-canola model (Robertson & Lilley, 2016) has been developed using the Plant Modelling Framework of Brown et al. (2014). All analyses and data gathering were conducted within the R software framework (R Core Team, 2022).

***Model validation and testing***

Observed flowering date, maturity date, length of the season, and yield were compared with the simulated counterpart from APSIM. Soil data was gathered from the Soil Survey Geographic (SSURGO) database (USDA-NRCS, 2022) and soil parameters were calculated using the function get_ssurgo_soil_profile. Daily long-term weather records (1986-2018) including maximum and minimum temperatures, precipitation, and solar radiation, were gathered from Iowa Environmental Mesonet (IEM) (ISU, 2022) using the function get_iem_apsim_met. Soil and weather records were gathered using the mentioned functions within apsimx package (Miguez, 2022) for each site coordinate (Table B1). Management practices employed (sowing date, irrigation, plant density, row spacing) were collected from the NWCVT (Table B1). The generic winter canola cultivar, available in the APSIM platform, was tested against many cultivars from the observed dataset.

Model performance was evaluated using the Coefficient of determination ($R^2$), Relative Root Mean Square Error (RRMSE), and Kling-Gupta model Efficiency (KGE) (Kling et al., 2012) using Metrica package (Correndo et al., 2022). Overall, crop phenology-related traits (flowering date, maturity date, and total season length) presented good model performance (Figure 5.1 A, B and C) with values of $R^2$ =0.81-0.89, RRMSE= 0.05-0.06, and KGE =0.89-0.90. Seed yield (Figure 5.1D) presented an inferior model performance ($R^2$= 0.59, RRMSE= 0.4, and KGE=0.62). This was expected due to the number of factors affecting this variable, and the large range of environmental variability from the observed dataset. Overall, the model failed to capture high yield potential environments, underestimating high yields (Figure 5.1D). Nevertheless, the model performance metrics were in accordance with previous reports (Wang et al., 2022), and the model was able to represent the general environmental quality (Figure 5.1D). Finally, this study highlights the utility of employing the generic winter canola cultivar to represent a wide range of genetic variability.

***Future weather assessment***

In order to evaluate the impact of the future climate on winter canola yield, APSIM-canola simulations were performed for all site-years, using the Climate Control manager included in the APSIM framework. Current weather was considered for the period (1997-2017). Temperature and precipitation changes were gathered from Almazroui et al. (2021) under two projected Shared Socio-economic Pathways (SSPs, SSP-2.6 and SSP-8.5) (Riahi et al., 2017); three future periods (i) Near (2021-2040), ii) Mid (2041-2060), and iii) Far (2080-2099)) and; two US sub-regions (i) Central North America (CNA) and ii) Eastern North America (ENA). Expected temperature and precipitations changes reported by Almazroui et al. (2021) are shown in Table 5.1. Standard management was set as the average of the practices employed in all the years of observed data for each site. This approach allowed to capture the site × management variations (Table B1)

***Spatial Clustering***

Sites were grouped into regions with similar future weather impacts using the spatial Fuzzy c-Means (FCM) clustering algorithm (Bezdek et al., 1984) with the function SFCMeans within the *geocmeans* (Gelb & Apparicio, 2021) R package. A linear regression analysis of the yield change across years (1997-2099) per site, was performed using the function *lmer* from the lme4 package (Bates et al., 2015a). The slope and intercept parameters from the regression model were the variables included in the clustering algorithm to classify the sites.

For each SSP, sites were grouped into three spatial clusters. Regardless of the SSP, cluster category names were denominated arbitrarily by the yield change trend across the 1997-

2099 period (slope) of the sites within each cluster. To explore the yield trends dissecting the future periods, the difference between the mean yield under current weather (1997-2017) and the three future periods (Near (2021-2040), Mid (2041-2060), and Far (2080-2099)) for each cluster was calculated under both SSPs.

## 5.3 Results

***Future weather assessment***

Predicted seed yield for the current, near, mid, and far future weather scenarios encompassed great variability among the 75 sites, ranging from 303 – 2766 kg $ha^{-1}$ for SSP 2.6 and 320 – 2758 kg $ha^{-1}$ for SSP 8.5. Minimum, $IQR_{25-75}$, median, mean, and maximum average values for each SSP and weather period combination are described in Table 5.2. Predicted seed yields for each site, SSP, and weather period combination are reported in Table B2. Furthermore, for each site and SSP, a linear regression model of yields across years was employed to quantify yield changes. The slope and intercept of the linear regression of each site were included in the fuzzy C-means clustering analysis.

***Potential areas for expansion***

Site-years were classified according to their future yield trend over time, obtaining four categories defined as i) positive (slope >0); ii) neutral (0 > slope > -1); iii) negative (-1 > slope > -6); and iv) high negative (slope < -6). The SSP 2.6 did not show high negative future yield trend sites, while the SSP 8.5 did not show neutral. Under the SSP 2.6, 44% of the sites presented positives trends and 51% neutral trends (Figure 5.2-A). Contrastingly, under the SSP 8.5, sites with positives trends increased to 80%, and 14% of the sites presented high negative trends (Figure 5.2-B).

Overall, regardless of the SSPs, winter canola yields increased in the central US Great Plains region, and a higher number of sites with negative yield trends are projected towards the west of the Southern Great Plains region despite of the SSPs and intensity (negative or high negative) (Figure 5.2). Specifically, the SSP 2.6 scenario resulted in sites with positive trends in the central US Great Plains region (Figure 5.2-A). Under the SSP 8.5 scenario (most extreme future weather conditions), the yield positive trends were observed in a larger area, expanded from the Great Plains into the Midwest, East of the Gulf Coast and the East Coastal regions (Figure 5.2-B).

Mean relative yield difference between the current and the three future weather periods (Figure 5.3) were similar for neutral and positive clusters (0.2 - 3%) under the SSP 2.6. The negative cluster presented higher variability (represented by the size of the box plots), especially on the mid (-11 %) and far (-12 %) future weather periods (Figure 5.3). Under the SSP 8.5, all clusters presented similar differences in the near future scenario (except with the high negative with more variation), and differences expanded as approaching far future weather (Figure 5.3). Overall, greater relative yield differences between near and far future resulted from the SSP 8.5, while SSP 2.6 presented more stable conditions. In the far future, sites under positive cluster yield trend had a yield increase of 15% for the SSP 2.6 and 37% for the SSP 8.5, each one of them relative to the negative cluster from each weather scenario (Figure 5.3).

## 5.4 Discussion

This study presented for the first-time changes in winter canola yield over time with the ability to identify potential areas for crop expansion within the US under projected future weather scenarios. The integration of a large multi-environment trial dataset with crop growth modeling and projected changes in weather scenarios permitted a summary of the overall magnitude and direction (negative, neutral, and positive) of winter canola yields under future conditions. The summary presented in this study could have important research and policy implications for winter canola production in the contiguous US region.

Previous studies from major global canola production regions (mainly Canada and Australia) explored in detail future climate pathways impacts on canola production (Qian et al., 2018; Xing et al., 2019). These studies considered scenarios without changes on future climate policy. This current study employed a broader view, using SSP projections, which also considered socioeconomic factors such as changes in population, economic growth, energy use practices, and technological development (Riahi et al., 2017). The direction of future climate impacts on crop yields depends on the environment, crop, and potential adaptation strategies (Zabel et al., 2021). The results of this study provide novel information, integrating the most recent climate modelling projections with crop model simulations under a broad environmental variation; and identifies potential suitable areas for winter canola production expansion.

Climate change is expected to compromise food security, leading to 11–33% of the population to face hunger by 2050, if no measures are taken (Hasegawa et al., 2021). Although overall trends of climate change impact on crop yields are negative, studies reported a large variation across the globe and different crops (Aggarwal et al., 2019; Challinor et al., 2014; Hasegawa et al., 2022). A global meta-analysis summarized that for North America, yields of major summer crops such as maize (*Zea mays* L.), soybeans (*Glycine max* (L.) Merr.) and rice

(*Oryza sativa*) will decrease, while winter wheat will increase due to climate change (Hasegawa et al., 2022). However, less efforts have been made to describe this trend on other winter crops such as canola (Wang et al., 2022). Results of this study agree with this positive trend of winter crops for the continental US region.

Future climate projections of higher rainfall during winter and spring months and increase of extreme heat and drought events during summer (Almazroui et al., 2021; Qian et al., 2018) could explain the different yield changes between summer and winter crops. Hence these projections indicate that an increased adoption of winter crops, such as winter canola, could be a feasible option to meet future food demand. Diversification of current cropping systems will be critical to cope with climate change uncertainties and increase crop resilience (Zsögön et al., 2022). This highlights the potential of winter canola to mitigate the expected food shortage driven by climate change, even under the most extreme conditions and in the far future.

The main limitation of this work is that changes in future atmospheric $CO_2$ concentration is not accounted for in this crop modeling exercise, which can also have an impact on canola yield. Future research should focus on i) determining the impact of leaf and canopy photosynthesis and include these estimates in crop growth models, ii) using seasonal weather data to predict future changes of the effects of timing and intensity of environmental stressors on yield and quality formation, and, iii) studying the oil formation process to include on canola crop growth models to better inform the climate driven changes of food supply and feedstocks for bio-diesel, and iv) exploring potential crop management options (e.g., changing sowing time, plant density, fertilizer needs) to mitigate future climate negative impacts or boost canola production under these new conditions.

## 5.5 Conclusions

This study showed that future weather projections are favorable for winter canola productivity in a large proportion of the US contiguous region. Sites on the Central-East Great Plains, Midwest, East of the Gulf Coast and the East Coast of the US were identified as sites with potential for higher positive canola yield gains. The SSP 2.6 presented more stable conditions, while with an average yield increase of 37%, winter canola yield gains were maximized under the SPP 8.5 most extreme climate conditions. This current study provides an insight on the potential geographical areas for canola crop expansion to satisfy future food and energy demands. In addition, it brings into attention the strategy of increasing winter crops production as a mitigation measure to future weather scenarios. However, this option will require considerations of regional consumers, government decisions, and adaptation of supply chain.

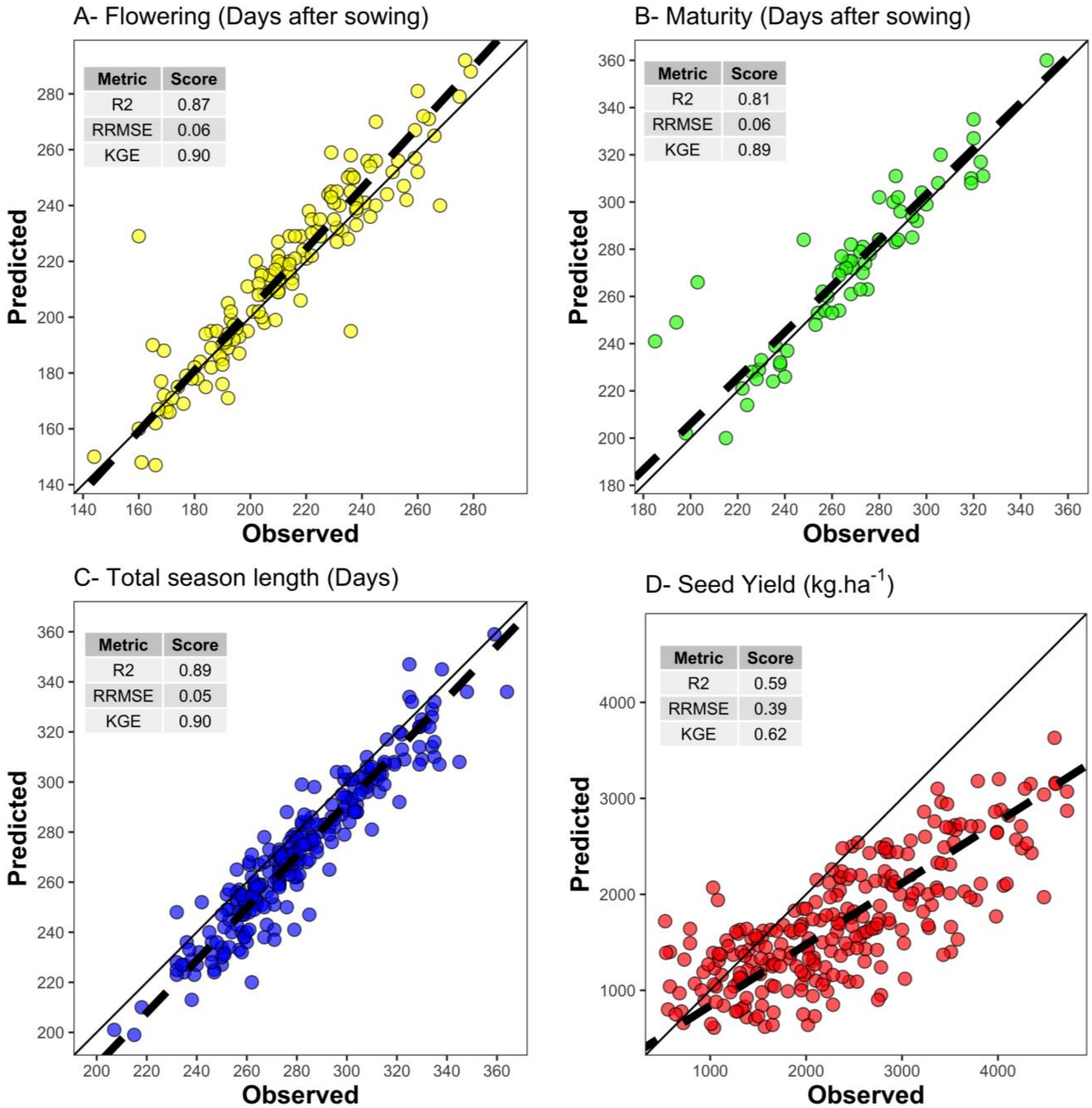


**Figure 5.1.** Observed versus predicted comparison for days after sowing to flowering (A) and from sowing to maturity (B), duration of the growing season (sowing until maturity) in days (C), and seed yield (D). In each panel, the solid line represents the 1:1 line, and the dashed line represents the linear model fitted with the observed vs predicted observations comparison. Metrics of each panel are Coefficient of determination ($R^2$), Relative Root Mean Square Error (RRMSE), and Kling-Gupta model Efficiency (KGE).

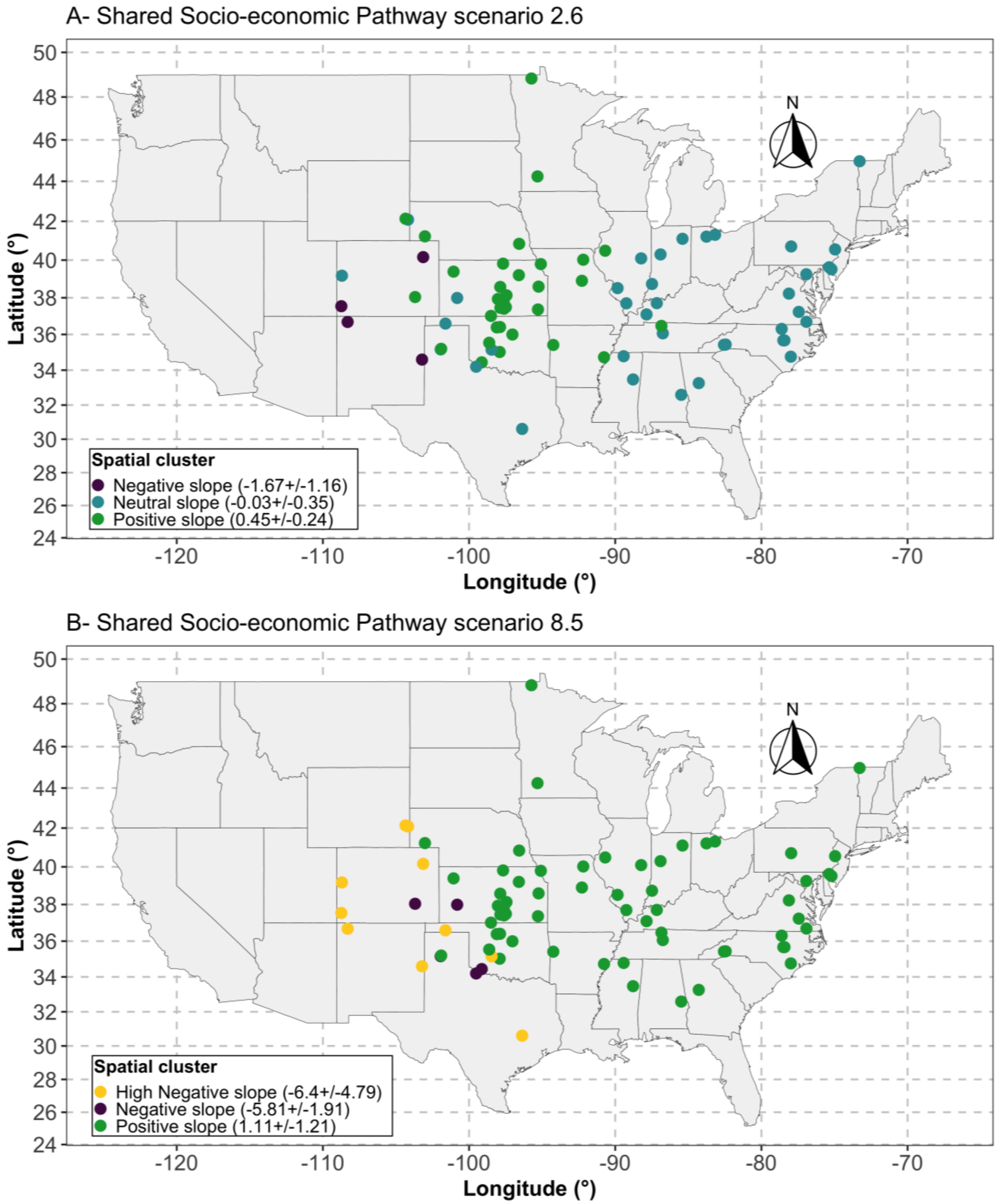


**Figure 5.2.** Spatial clusters yield trends for the 1997-2099 period in Central and East US regions. Points represent each site. Panel A shows trends under the Shared Socio-economic Pathway 2.6 and Panel B under 8.5. Cluster categories were i) positive (slope >0, green points); ii) neutral (0 > slope > -1, light blue points); iii) negative ( -1 > slope > -6, purple points); and iv) high negative (slope <-6, yellow points). Map lines delineate study areas and do not necessarily depict accepted national boundaries.

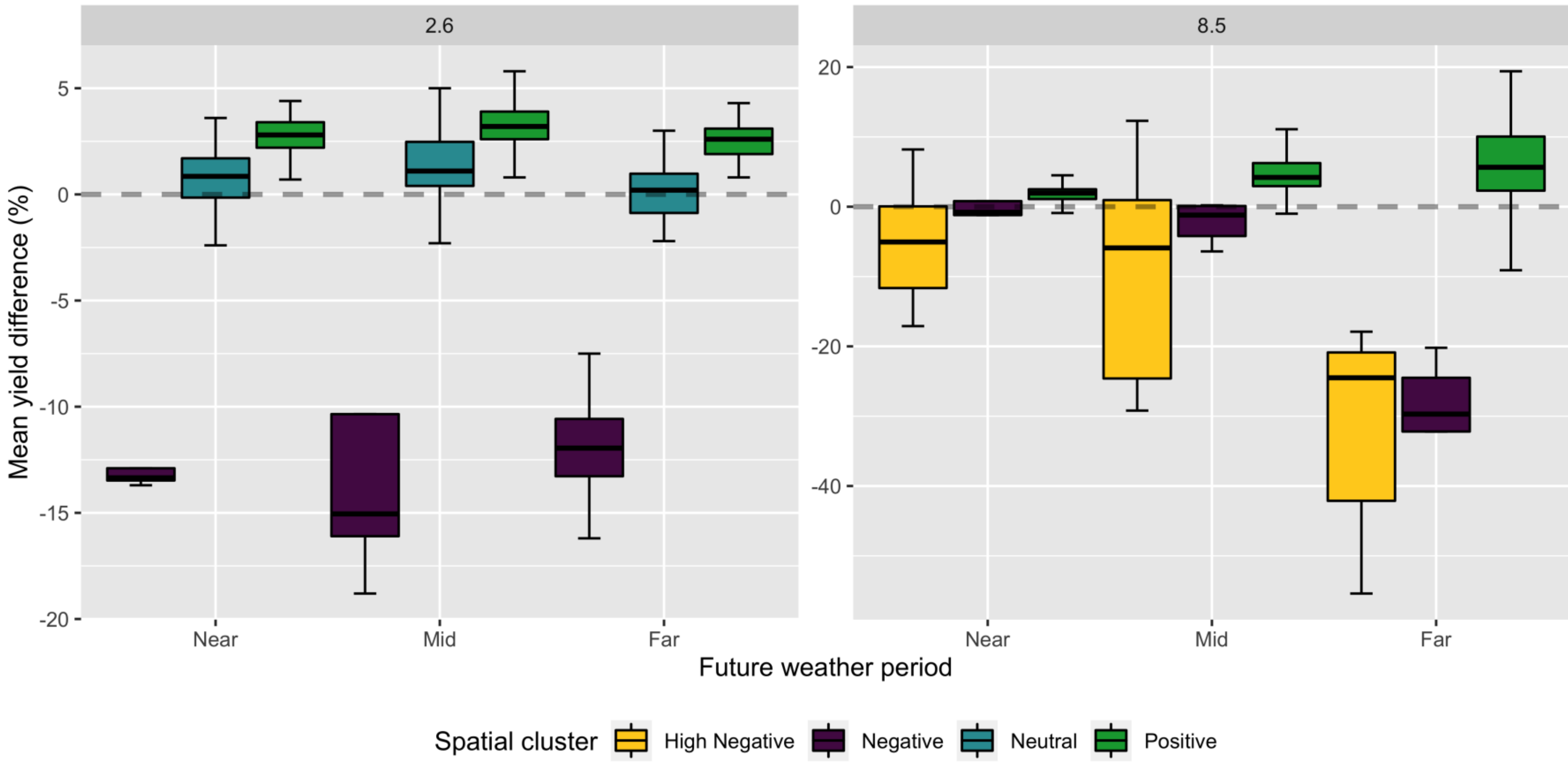


**Figure 5.3.** Boxplots of mean relative yield difference (%) of future weather to current weather for each spatial cluster and for both Shared Socio-economic Pathways SSPs 2.6 and 8.5. Cluster categories were i) positive (slope >0, green boxes); ii) neutral (0 > slope > -1, light blue boxes); iii) negative ( -1 > slope > -6, purple boxes); and iv) high negative (slope <-6, yellow boxes). A) RCP 2; B) RCP8. Weather periods were current weather (1997-2017) and the three future periods, Near (2021-2040), Mid (2041-2060), and Far (2080-2099). Boxplots portray the 25th (lower hinge), 50th (solid black line), and 75th (upper hinge) percentiles, largest value no further than 1.5 inter-quartile range (lower whisker), smallest value at most 1.5 inter-quartile range (upper whisker), and outlying observations (points). Dashed horizontal line represents the 0 mean difference level.

**Table 5.1.**Reported changes for two projected Shared Socio-economic Pathways (SSPs, SSP-2.6 and SSP-8.5); three future periods, Near (2021-2040), Mid (2041-2060), and Far (2080-2099) and; two US sub-regions, Central North America (CNA) and Eastern North America (ENA). Adapted from Almazroui et al. (2021).

| | | **CNA sub-region** | | | **ENA sub-region** | | |
|---|---|---|---|---|---|---|---|
| | **Scenario** | **Near** | **Mid** | **Far** | **Near** | **Mid** | **Far** |
| **Temperature (°C)** | SSP-2.6 | 1.29 | 1.58 | 1.67 | 1.13 | 1.55 | 1.59 |
| | SSP-8.5 | 1.29 | 2.58 | 5.53 | 1.23 | 2.63 | 5.33 |
| **Precipitation (%)** | SSP-2.6 | 0.85 | 2.14 | 1.77 | 3.58 | 3.86 | 5.36 |
| | SSP-8.5 | 2.02 | 1.89 | 4.41 | 3.67 | 5.65 | 11.37 |

**Table 5.2.** Summary minimum, interquartile range $IQR_{25\text{-}75}$, median, mean, and maximum average yield values (kg $ha^{-1}$). Shared Socio-economic Pathways (SSPs, SSP-2.6 and SSP-8.5). Weather periods: Current (1997-2017), Near (2021-2040), Mid (2041-2060), and Far (2080-2099).

| | | SSP-2.6 | | | SSP-8.5 | | |
|---|---|---|---|---|---|---|---|
| **Weather** | **Current** | **Near** | **Mid** | **Far** | **Near** | **Mid** | **Far** |
| Period | 1997-2017 | 2021-2040 | 2041-2060 | 2080-2099 | 2021-2040 | 2041-2060 | 2080-2099 |
| Minimum | 404 | 356 | 303 | 373 | 377 | 429 | 320 |
| $IQR_{25\text{-}75}$ | 1117-1880 | 1147-1950 | 1158-1961 | 1146-1933 | 1146-1965 | 1159-1938 | 1101-1910 |
| Median | 1516 | 1513 | 1515 | 1506 | 1504 | 1545 | 1520 |
| Mean | 1540 | 1555 | 1561 | 1549 | 1555 | 1571 | 1514 |
| Maximum | 2691 | 2761 | 2766 | 2755 | 2753 | 2758 | 2659 |

# Chapter 6 - Final remarks

This dissertation provides a novel summary of the great potential of winter canola production expansion. Research presented in this dissertation contributes foundational knowledge of how current and future expected challenges on the current agricultural system, with special focus on the second most relevant oilseed crop currently produced.

In Chapters 2 and 3, we have documented the main environmental variables that define two of the major challenges that face winter canola production. Chapter 2 highlighted that minimum temperature, fluctuations above or below 0°C, and wind chill temperatures highly affected winter canola survival. Diverse temperature fluctuations in the continental US Great Plains seem to trigger phases of dormancy and re-growth, creating significant stress on the plant. Chapter 3 identified that precipitation and photothermal coefficient during early and mid-stages of the seed filling period, determined most of the seed oil concentration variability. Using long-term multi-environment trials (METs) with weather auxiliary data, both studies demonstrate that there is a large geographical area in the US with potential for medium and high winter canola survival and seed oil concentration. Findings suggest further efforts on variety development with high winter-kill tolerance, seed yield and oil concentration. Outcomes from Chapters 2 and 3 provide essential information that can assist canola breeding processes to select varieties better adapted to cold environments and with stability for both yield and oil performance.

Chapter 4 global meta-analysis addresses the unclear response reported in the literature with contrasting results on the effects of abiotic stresses in canola seed yield and quality. Results quantified canola seed productivity and quality responses to short, long, early, and late heat and drought stresses. Findings summarized that heat stress reduces seed oil concentration, yield, and oil yield while increasing seed protein concentration. Drought stress has neutral effects on seed oil and protein concentration but reduces final canola seed and oil yield. In context of future climate change affecting canola production (Ray et al., 2019), chapter 4 provides critical information dissecting the magnitude and direction of abiotic stresses impact on canola productivity and quality. The indeterminate growth habit of canola can explain some of the different results previously reported. Under some type of stresses, canola has a “compensation” mechanism producing more flowers and branches. This highlights the importance of identifying stress timing and duration with highest impacts when the crop is not able to compensate negative stress effects. Investigations from this chapter can be used in future research and breeding programs to mitigate unfavorable future climate scenarios.

Lastly, chapter 5 presented changes in winter canola yield over time and identifies potential areas for crop expansion within the US under projected future weather scenarios. Not only does this chapter integrate METs and climate data using canola growth simulations models, but also includes how future climate change could be considering socio-economic factors. The most recent climate modelling projects, known as the Coupled Model Intercomparison Project version 6 (CMIP6), are recently being used for the Intergovernmental Panel on Climate Change (IPCC) Sixth Assessment Report. The analysis presented in Chapter 5 used a broader view from the shared socioeconomic pathways (SSPs) narratives, which define if future emissions reductions could or could not be achieved. These projections integrate different levels of radiative forcings and greenhouse gases as consequence of climate change with other factors such as changes in population, economic growth, energy use practices, and technological development (Riahi et al., 2017). Results from chapter 5 documented those future projections will favor winter canola production with yield gains in a large area of the US, highlighting the relevance of this research on potential future policy implications for the expansion of winter canola production.

Some of the main research limitations throughout this dissertation were: i) the subjectivity of winter survival evaluation as a visual rating; ii) the use of gridded weather data, that could lead to less accurate estimations; iii) the lack of completeness (and data balanced) for all canola varieties, not all varieties were tested over all sites-years on Chapters 2 and 3, reducing the power to understand the contribution of the G component to the variation in winter survival and seed oil concentration at spatio-temporal scales; iv) the use of only imposed (controlled studies) abiotic stress treatments in Chapter 4, reducing the understating of stress impacts under normal field production conditions; v) the lack of exploration of different mitigation practices to avoid heat and drought stresses under critical periods; and vi) the lack of consideration of potential changes in atmospheric $CO_2$ concentration that could also have an impact on the final winter canola yield.

Future research should focus on better identifying "phenotypes" to improve winter survival, with more quantitative measurements, increasing standardization and precision. More resources should be allocated to understand the physiological processes and the interaction with factors such as meteorology, management, and genotype; that determine winter survival and final oil yield. Stand establishment, silique shattering, harvesting, and proximity of seed delivery point challenges should be addressed to incorporate winter canola in producers' farming system. With an increased demand for healthy edible oils, the impact of stresses on oil quality (fatty acid profile) should be further explored. Lastly, accurate seed oil predictive models are needed to further extend our knowledge of climate change's impact on food and bio-diesel future supply.

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# Appendix A - Chapter 2 Supplementary Tables

**Table A.1.** Genotype groups information.

| Genotype group | Name | Decade of release | Type |
|---|---|---|---|
| Tolerant | Celsius | 2000s | Open-pollinated |
| | Explus | 2010s | Hybrid |
| | KS2098 | 2000s | Open-pollinated |
| | KS2169 | 2000s | Open-pollinated |
| | KS9012 | 2000s | Open-pollinated |
| | Torrington | 2010s | Open-pollinated |
| | USI2002 | 2000s | Open-pollinated |
| Semi-tolerant | 15.WC.05633 | 2010s | Open-pollinated |
| | 15.WD.1 | 2010s | Open-pollinated |
| | 45D03 | 2000s | Hybrid |
| | 46W14 | 2000s | Hybrid |
| | 46W99 | 2010s | Hybrid |
| | AAMU-33-07 | 2010s | Open-pollinated |
| | Alabaster | 2010s | Hybrid |
| | ARC00004-2 | 2000s | Open-pollinated |
| | ARC00005-2 | 2000s | Open-pollinated |
| | ARC00024-2 | 2000s | Open-pollinated |
| | ARC2189-1 | 1990s | Open-pollinated |
| | ARC2189-2 | 1990s | Open-pollinated |
| | ARC91019-50-e2 | 2000s | Open-pollinated |
| | ARC97019 | 2000s | Open-pollinated |
| | ARC98007 | 2000s | Open-pollinated |
| | Argos | 2010s | Hybrid |
| | Artoga | 2010s | Hybrid |
| | Atora | 2010s | Hybrid |
| | Banjo | 2000s | Hybrid |
| | Casino | 1990s | Open-pollinated |
| | CHH2311 | 2010s | Hybrid |
| | CWH042 | 2010s | Hybrid |
| | CWH081 | 2000s | Hybrid |
| | CWH095 | 2000s | Hybrid |
| | CWH111 | 2000s | Hybrid |
| | CWH116 | 2000s | Hybrid |
| | CWH633 | 2000s | Open-pollinated |
| | DK Exstorm | 2010s | Hybrid |
| | DK Imiron CL | 2010s | Hybrid |
| | DK Imistar CL | 2010s | Hybrid |
| | DK Imiron CL | 2010s | Hybrid |
| | DK Sensei | 2010s | Hybrid |
| | DKW13-69 | 2000s | Open-pollinated |
| | DKW44-10 | 2000s | Open-pollinated |
| | DKW45-25 | 2010s | Open-pollinated |
| | DKW47-15 | 2000s | Open-pollinated |
| | DSV05103 | 2000s | Hybrid |
| | DSV05104 | 2000s | Hybrid |
| | DSV07100 | 2000s | Hybrid |
| | Dynastie | 2010s | Hybrid |
| | Extra | 2010s | Hybrid |
| | Falstaff | 2000s | Open-pollinated |
| | Forza | 2000s | Open-pollinated |
| | Garou | 2010s | Hybrid |
| | Gospel | 2000s | Hybrid |
| | Griffin | 2010s | Open-pollinated |

| | | |
|---|---|---|
| Hamour | 2010s | Hybrid |
| Hornet | 2000s | Hybrid |
| HPX-6271 | 2010s | Open-pollinated |
| HPX-6406 | 2010s | Open-pollinated |
| HPX-7228 | 2010s | Open-pollinated |
| HyCLASS107W | 2000s | Open-pollinated |
| HyCLASS110W | 2000s | Open-pollinated |
| HyCLASS125W | 2010s | Open-pollinated |
| HyCLASS225W | 2010s | Open-pollinated |
| Kadore | 2010s | Open-pollinated |
| KS2004 | 2000s | Open-pollinated |
| KS2064 | 2000s | Open-pollinated |
| KS2185 | 2000s | Open-pollinated |
| KS2427 | 2000s | Open-pollinated |
| KS3018 | 2000s | Open-pollinated |
| KS3067 | 2000s | Open-pollinated |
| KS3068 | 2000s | Open-pollinated |
| KS3074 | 2000s | Open-pollinated |
| KS3077 | 2000s | Open-pollinated |
| KS3132 | 2000s | Open-pollinated |
| KS3254 | 2000s | Open-pollinated |
| KS3350 | 2000s | Open-pollinated |
| KS4085 | 2010s | Open-pollinated |
| KS4428 | 2010s | Open-pollinated |
| KS8285 | 2000s | Open-pollinated |
| KS8367 | 2000s | Open-pollinated |
| KS9124 | 2000s | Open-pollinated |
| KS9183 | 2000s | Open-pollinated |
| KSR07352S | 2010s | Open-pollinated |
| KSR07363 | 2010s | Open-pollinated |
| KSR4653S | 2010s | Open-pollinated |
| KSUR1211 | 2010s | Open-pollinated |
| KSUR21 | 2010s | Open-pollinated |
| Kuga | 2010s | Hybrid |
| Maestro | 2000s | Hybrid |
| MH 09DJ058 | 2010s | Hybrid |
| MH 07J14 | 2010s | Hybrid |
| MH 10G11 | 2010s | Hybrid |
| MH 10L23 | 2010s | Hybrid |
| NK Petrol | 2010s | Hybrid |
| NK Technic | 2010s | Hybrid |
| NPZ0326 | 2000s | Hybrid |
| NZ0404 | 2000s | Hybrid |
| NPZ0591RR | 2000s | Hybrid |
| NPZ0791RR | 2000s | Hybrid |
| Ovation | 2000S | Open-pollinated |
| Phoenix CL | 2010s | Hybrid |
| Plurax CL | 2010s | Hybrid |
| Popular | 2010s | Hybrid |
| Quartz | 2010s | Open-pollinated |
| Raffiness | 2010s | Hybrid |
| Rally | 2000s | Hybrid |
| Riley | 2010s | Open-pollinated |
| Rumba | 2010s | Open-pollinated |
| Safran | 2010s | Open-pollinated |
| Satori | 2000s | Hybrid |
| Sitro | 2000s | Hybrid |
| SLM0402 | 2000s | Hybrid |
| Star 915w | 2010s | Open-pollinated |

| | | | |
|---|---|---|---|
| | SW 013022 | 2000s | Open-pollinated |
| | SW 013121 | 2000s | Open-pollinated |
| | SW 013173 | 2000s | Open-pollinated |
| | SW 013211 | 2000s | Open-pollinated |
| | SW 013253 | 2000s | Open-pollinated |
| | SY Saveo | 2010s | Hybrid |
| | Talent | 2000s | Hybrid |
| | TCI Exp 983 | 2010s | Open-pollinated |
| | TCI.06.M1 | 2000s | Open-pollinated |
| | TCI.06.M3 | 2000s | Open-pollinated |
| | TCI.06.M4 | 2000s | Open-pollinated |
| | Visby | 2010s | Hybrid |
| | VSX-3 | 2010s | Open-pollinated |
| | WC.15.7.5 | 2010s | Open-pollinated |
| | WC.9.7.5.7 | 2010s | Open-pollinated |
| | X01W692C | 2010s | Hybrid |
| | X02W534C | 2010s | Hybrid |
| | X10W443C | 2010s | Hybrid |
| | X10W665C | 2010s | Hybrid |
| | X12W377C | 2010s | Hybrid |
| | X12W447C | 2010s | Hybrid |
| | X13W029C | 2010s | Hybrid |
| Semi-susceptible | 46W94 | 2000s | Hybrid |
| | AAMU-18-07 | 2000s | Open-pollinated |
| | Abilene | 1990s | Open-pollinated |
| | ARC2180-1 | 2000s | Open-pollinated |
| | ARC90016-pr377 | 2000s | Open-pollinated |
| | ARC92004-1 | 2000s | Open-pollinated |
| | ARC92007-2 | 2000s | Open-pollinated |
| | ARC97018 | 2000s | Open-pollinated |
| | Baldur | 2000s | Hybrid |
| | Baros | 2000s | Open-pollinated |
| | Ceres | 1990s | Open-pollinated |
| | Chrome | 2010s | Open-pollinated |
| | Claremore | 2000s | Open-pollinated |
| | Dimension | 2000s | Hybrid |
| | DK Sensei | 2010s | Hybrid |
| | DK Severnyi | 2010s | Hybrid |
| | DKW13-62 | 2000s | Open-pollinated |
| | DKW41-10 | 2000s | Open-pollinated |
| | DKW46-15 | 2000s | Open-pollinated |
| | Edimax CL | 2010s | Hybrid |
| | Einstein | 2010s | Hybrid |
| | Flash | 2000s | Hybrid |
| | Hekip | 2010s | Hybrid |
| | Hidylle | 2010s | Hybrid |
| | Hybrigold | 2010s | Hybrid |
| | Hybristar | 2010s | Hybrid |
| | Hybrisurf | 2010s | Hybrid |
| | HyCLASS115W | 2000s | Open-pollinated |
| | HyCLASS154W | 2000s | Open-pollinated |
| | Inspiration | 2010s | Hybrid |
| | Jetton | 1990s | Open-pollinated |
| | Kalif | 2000s | Open-pollinated |
| | Kiowa | 2000s | Open-pollinated |
| | Kronos | 1990s | Hybrid |
| | KS3302 | 2000s | Open-pollinated |
| | KS7436 | 2000s | Open-pollinated |
| | Mercedes | 2010s | Hybrid |

| | | | |
|---|---|---|---|
| | MH 12AY04 | 2010s | Hybrid |
| | MH 12AY27 | 2010s | Hybrid |
| | MH 12AY36 | 2010s | Hybrid |
| | MH 06E10 | 2010s | Hybrid |
| | MH 09E3 | 2010s | Hybrid |
| | Plainsman | 1990s | Open-pollinated |
| | PT211 | 2010s | Hybrid |
| | Rasmus | 2000s | Open-pollinated |
| | Sumner | 2000s | Open-pollinated |
| | SY Marten | 2010s | Hybrid |
| | Taurus | 2000s | Hybrid |
| | Titan | 2000s | Hybrid |
| | Trabant | 2000s | Hybrid |
| | Viking | 2000s | Open-pollinated |
| | Virginia | 2000s | Open-pollinated |
| | VSX-2 | 2000s | Open-pollinated |
| | Wichita | 1990s | Open-pollinated |
| | Wotan | 2000s | Open-pollinated |
| | X01W522C | 2000s | Hybrid |
| Susceptible | Albatros | 2010s | Hybrid |
| | ARC98015 | 2000s | Open-pollinated |
| | DKW13-54 | 2000s | Open-pollinated |
| | DKW13-86 | 2000s | Open-pollinated |
| | DSV06201 | 2000s | Hybrid |
| | DSV06202 | 2000s | Hybrid |
| | HPX-567 | 2000s | Open-pollinated |
| | KS7436-055 | 2000s | Open-pollinated |
| | MH 09H19 | 2010s | Hybrid |
| | TCI.06.M2 | 2000s | Open-pollinated |

# Appendix B - Chapter 5 Supplementary Tables

**Table B.1.** Average management practices of all years per site. Site specific information observed: city and state name, coordinates, region (Central North America (CNA) and Eastern North America (ENA)), irrigation, fertilization, plant population and row spacing.

| City | State | Latitude | Longitude | Region | Sowing date | Irrig. at planting (mm) | Irrig. in Spring (mm) | N at planting (kg ha-1) | N in spring (kg ha-1) | Plant pop. (pl m$^2$) | Row spacing (cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Auburn | Alabama | 32.59 | -85.49 | ENA | 12-nov | 0 | 7 | 40 | 77 | 106 | 178 |
| Kibler | Arkansas | 35.42 | -94.24 | CNA | 3-oct | 0 | 0 | 40 | 77 | 160 | 178 |
| Marianna | | 34.73 | -90.77 | CNA | 24-sep | 0 | 0 | 60 | 116 | 170 | 178 |
| Akron | Colorado | 40.16 | -103.14 | CNA | 19-aug | 0 | 33 | 40 | 77 | 60 | 254 |
| Fruita | | 39.18 | -108.70 | CNA | 13-sep | 0 | 0 | 60 | 116 | 139 | 762 |
| Rocky ford | | 38.04 | -103.69 | CNA | 18-aug | 0 | 330 | 40 | 77 | 90 | 210 |
| Yellow jacket | | 37.54 | -108.74 | CNA | 13-sep | 0 | 91 | 40 | 77 | 115 | 267 |
| Griffin | Georgia | 33.26 | -84.29 | ENA | 10-oct | 0 | 0 | 60 | 116 | 110 | 178 |
| Belleville | Illinois | 38.52 | -89.84 | ENA | 17-sep | 0 | 0 | 60 | 116 | 195 | 190 |
| Carbondale | | 37.70 | -89.24 | ENA | 15-sep | 0 | 0 | 50 | 96 | 190 | 199 |
| Macomb | | 40.49 | -90.69 | CNA | 18-sep | 0 | 0 | 40 | 77 | 122 | 184 |
| Urbana | | 40.09 | -88.23 | ENA | 10-sep | 0 | 0 | 50 | 96 | 65 | 190 |
| Columbia city | Indiana | 41.11 | -85.40 | ENA | 9-sep | 0 | 0 | 40 | 77 | 82 | 152 |
| Throckmorton | | 40.30 | -86.90 | ENA | 15-sep | 0 | 0 | 40 | 77 | 142 | 152 |
| Vincennes | | 38.74 | -87.49 | ENA | 18-sep | 3 | 0 | 50 | 96 | 97 | 158 |
| Andale | Kansas | 37.79 | -97.63 | CNA | 23-sep | 0 | 0 | 50 | 96 | 90 | 229 |
| Belleville | | 39.81 | -97.67 | CNA | 12-sep | 0 | 0 | 50 | 96 | 87 | 235 |
| Colby | | 39.39 | -101.06 | CNA | 14-aug | 28 | 54 | 30 | 58 | 73 | 419 |
| Clearwater | | 37.49 | -97.48 | CNA | 24-sep | 0 | 0 | 40 | 77 | 142 | 203 |
| Conway springs | | 37.41 | -97.61 | CNA | 14-oct | 0 | 0 | 40 | 77 | 72 | 254 |
| Garden city | | 37.99 | -100.81 | CNA | 18-sep | 0 | 234 | 40 | 77 | 109 | 237 |
| Hutchinson | | 37.93 | -98.03 | CNA | 21-sep | 0 | 0 | 40 | 77 | 95 | 224 |
| Kiowa | | 37.02 | -98.50 | CNA | 26-sep | 0 | 0 | 60 | 116 | 106 | 246 |
| Manhattan | | 39.21 | -96.59 | CNA | 17-sep | 0 | 0 | 40 | 77 | 87 | 237 |
| Norwich | | 37.46 | -97.86 | CNA | 24-sep | 0 | 0 | 40 | 77 | 96 | 254 |
| Ottawa | | 38.60 | -95.24 | CNA | 10-sep | 0 | 0 | 30 | 58 | 140 | 152 |
| Parsons | | 37.37 | -95.29 | CNA | 10-sep | 0 | 0 | 40 | 77 | 98 | 178 |
| Marquette | | 38.58 | -97.87 | CNA | 22-sep | 0 | 0 | 40 | 77 | 123 | 220 |
| Hesston | | 38.13 | -97.44 | CNA | 14-sep | 0 | 0 | 30 | 58 | 91 | 229 |
| Troy | | 39.79 | -95.09 | CNA | 21-sep | 0 | 0 | 50 | 96 | 124 | 210 |
| Princeton | Kentucky | 37.10 | -87.86 | ENA | 3-oct | 0 | 0 | 60 | 116 | 142 | 178 |
| Russellville | | 37.71 | -87.16 | ENA | 24-sep | 0 | 0 | 30 | 58 | 128 | 190 |
| Beltsville | Maryland | 39.25 | -76.93 | ENA | 18-sep | 0 | 0 | 30 | 58 | 124 | 190 |
| Lamberton | Minnesota | 44.24 | -95.32 | CNA | 28-aug | 0 | 0 | 30 | 58 | 142 | 190 |
| Roseau | | 48.84 | -95.73 | CNA | 30-aug | 0 | 0 | 30 | 58 | 60 | 152 |
| Columbia | Missouri | 38.91 | -92.28 | CNA | 10-sep | 0 | 0 | 30 | 58 | 115 | 188 |
| Novelty | | 40.02 | -92.19 | CNA | 2-sep | 0 | 0 | 40 | 77 | 165 | 190 |
| Holly springs | Mississippi | 34.79 | -89.43 | ENA | 17-oct | 0 | 0 | 40 | 77 | 127 | 190 |
| Starkville | | 33.47 | -88.78 | ENA | 25-sep | 0 | 0 | 30 | 58 | 126 | 178 |
| Clayton | North Carolina | 35.67 | -78.42 | ENA | 4-oct | 0 | 0 | 30 | 58 | 127 | 152 |
| Fletcher | | 35.44 | -82.45 | ENA | 20-sep | 0 | 0 | 30 | 58 | 142 | 178 |
| Mills river | | 35.43 | -82.56 | ENA | 3-oct | 0 | 0 | 50 | 96 | 142 | 190 |
| Oxford | | 36.31 | -78.61 | ENA | 9-oct | 0 | 0 | 30 | 58 | 142 | 203 |
| Raleigh | | 35.67 | -78.50 | ENA | 16-oct | 0 | 0 | 30 | 58 | 142 | 203 |
| Wallace | | 34.76 | -77.99 | ENA | 17-oct | 0 | 0 | 50 | 96 | 142 | 200 |
| Williamsdale | | 34.76 | -77.99 | ENA | 17-oct | 0 | 0 | 50 | 96 | 142 | 200 |
| Lincoln | Nebraska | 40.85 | -96.57 | CNA | 13-sep | 0 | 0 | 60 | 116 | 94 | 229 |
| Sidney | | 41.23 | -103.02 | CNA | 10-sep | 46 | 33 | 50 | 96 | 84 | 305 |
| Centerton | New | 39.52 | -75.21 | ENA | 10-sep | 0 | 0 | 40 | 77 | 89 | 210 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pittstown | Jersey | 40.56 | -74.96 | ENA | 20-sep | 0 | 0 | 50 | 96 | 73 | 229 |
| Woodstown | | 39.63 | -75.36 | ENA | 21-sep | 0 | 0 | 70 | 135 | 150 | 210 |
| Clovis | New Mexico | 34.60 | -103.22 | CNA | 15-sep | 0 | 330 | 60 | 116 | 110 | 152 |
| Farmington | | 36.69 | -108.31 | CNA | 6-sep | 0 | 699 | 80 | 155 | 123 | 254 |
| Custar | Ohio | 41.22 | -83.76 | ENA | 6-sep | 0 | 0 | 60 | 116 | 149 | 178 |
| Fremont | | 41.31 | -83.17 | ENA | 10-sep | 0 | 0 | 50 | 96 | 141 | 178 |
| Chickasha | Oklahoma | 35.02 | -97.91 | CNA | 22-sep | 0 | 0 | 40 | 77 | 80 | 224 |
| Enid | | 36.41 | -97.88 | CNA | 21-sep | 0 | 0 | 30 | 58 | 92 | 219 |
| Fort cobb | | 35.15 | -98.46 | CNA | 30-sep | 0 | 0 | 60 | 116 | 142 | 190 |
| Goodwell | | 36.60 | -101.62 | CNA | 20-sep | 14 | 117 | 50 | 96 | 81 | 203 |
| Lahoma | | 36.39 | -98.11 | CNA | 21-sep | 0 | 0 | 30 | 58 | 102 | 220 |
| Perkins | | 35.99 | -97.03 | CNA | 23-sep | 0 | 0 | 30 | 58 | 90 | 220 |
| Tipton | | 34.44 | -99.13 | CNA | 20-sep | 0 | 0 | 50 | 96 | 96 | 199 |
| Weatherford | | 35.54 | -98.62 | CNA | 24-sep | 0 | 0 | 30 | 58 | 121 | 229 |
| State college | Pennsylvania | 40.71 | -77.96 | ENA | 12-sep | 0 | 0 | 60 | 116 | 123 | 178 |
| Nashville | Tennessee | 36.06 | -86.75 | ENA | 19-sep | 0 | 0 | 40 | 77 | 62 | 190 |
| Springfield | | 36.47 | -86.84 | ENA | 17-oct | 0 | 0 | 50 | 96 | 168 | 178 |
| Spring hill | | 36.47 | -86.84 | ENA | 17-oct | 0 | 0 | 50 | 96 | 168 | 178 |
| Amarillo | Texas | 35.17 | -101.94 | CNA | 27-sep | 86 | 0 | 40 | 77 | 95 | 210 |
| Bushland | | 35.20 | -101.91 | CNA | 14-sep | 0 | 64 | 30 | 58 | 60 | 762 |
| Chillicothe | | 34.19 | -99.52 | CNA | 16-oct | 62 | 20 | 40 | 77 | 84 | 254 |
| College station | | 30.61 | -96.37 | CNA | 23-oct | 0 | 36 | 30 | 58 | 136 | 210 |
| Orange | Virginia | 38.22 | -78.12 | ENA | 19-sep | 0 | 0 | 50 | 96 | 106 | 186 |
| Petersburg | | 37.24 | -77.44 | ENA | 9-oct | 0 | 0 | 30 | 58 | 166 | 367 |
| Suffolk | | 36.69 | -76.92 | ENA | 2-oct | 0 | 0 | 40 | 77 | 171 | 305 |
| Alburgh | Vermont | 44.98 | -73.28 | ENA | 16-aug | 0 | 0 | 30 | 58 | 88 | 152 |
| Torrington | Wyoming | 42.13 | -104.35 | CNA | 22-aug | 252 | 152 | 40 | 77 | 95 | 210 |

**Table B.2.** City and state specific predicted winter canola yield (kg $ha^{-1}$) under current (1997-2017), and future Near (2021-2040), Mid (2041-2060), and Far (2080-2099) weather scenarios for both Shared Socio-economic pathways (SSPs, SSP 2.6, SSP 8.5).

| | | | **SSP 2.6** | | | **SSP 8.5** | | |
|---|---|---|---|---|---|---|---|---|
| **City** | **State** | **Current** | **Near** | **Mid** | **Far** | **Near** | **Mid** | **Far** |
| Auburn | Alabama | 1097 | 1117 | 1129 | 1105 | 1120 | 1146 | 1108 |
| Kibler | Arkansas | 1538 | 1572 | 1570 | 1567 | 1565 | 1601 | 1636 |
| Marianna | | 2098 | 2173 | 2169 | 2166 | 2164 | 2190 | 2164 |
| Akron | Colorado | 1637 | 1414 | 1330 | 1372 | 1381 | 1159 | 730 |
| Fruita | | 457 | 426 | 303 | 374 | 379 | 429 | 375 |
| Rocky ford | | 856 | 873 | 952 | 893 | 899 | 846 | 453 |
| Yellow jacket | | 1206 | 1045 | 1027 | 1058 | 1062 | 952 | 901 |
| Griffin | Georgia | 2029 | 2049 | 2050 | 2036 | 2051 | 2052 | 1994 |
| Belleville | Illinois | 2334 | 2374 | 2404 | 2358 | 2381 | 2451 | 2438 |
| Carbondale | | 1649 | 1673 | 1690 | 1663 | 1678 | 1740 | 1802 |
| Macomb | | 1680 | 1730 | 1735 | 1725 | 1723 | 1793 | 1910 |
| Urbana | | 2181 | 2203 | 2215 | 2186 | 2199 | 2265 | 2315 |
| Columbia city | Indiana | 1380 | 1409 | 1412 | 1400 | 1408 | 1421 | 1455 |
| Throckmorton | | 1563 | 1570 | 1580 | 1562 | 1572 | 1619 | 1698 |
| Vincennes | | 1314 | 1325 | 1334 | 1310 | 1326 | 1338 | 1342 |
| Andale | Kansas | 2051 | 2109 | 2104 | 2103 | 2101 | 2129 | 2169 |
| Belleville | | 2078 | 2133 | 2134 | 2126 | 2125 | 2165 | 2187 |
| Colby | | 1081 | 1150 | 1158 | 1148 | 1148 | 1179 | 1222 |
| Clearwater | | 1613 | 1649 | 1655 | 1644 | 1643 | 1680 | 1715 |
| Conway | | 1560 | 1618 | 1612 | 1609 | 1606 | 1609 | 1558 |
| Garden city | | 1549 | 1524 | 1528 | 1532 | 1536 | 1450 | 1235 |
| Hutchinson | | 1668 | 1722 | 1729 | 1716 | 1714 | 1766 | 1779 |
| Kiowa | | 2691 | 2761 | 2766 | 2755 | 2753 | 2758 | 2470 |
| Manhattan | | 1607 | 1661 | 1668 | 1656 | 1654 | 1723 | 1801 |
| Norwich | | 1516 | 1544 | 1539 | 1537 | 1535 | 1578 | 1564 |
| Ottawa | | 1302 | 1343 | 1351 | 1337 | 1335 | 1407 | 1520 |
| Parsons | | 1464 | 1513 | 1521 | 1506 | 1504 | 1578 | 1700 |
| Marquette | | 1960 | 2011 | 2019 | 2006 | 2005 | 2066 | 2137 |
| Hesston | | 1307 | 1345 | 1348 | 1340 | 1339 | 1399 | 1474 |
| Troy | | 1818 | 1847 | 1856 | 1842 | 1841 | 1926 | 2046 |
| Princeton | Kentucky | 2387 | 2435 | 2466 | 2420 | 2440 | 2506 | 2408 |
| Russellville | | 1047 | 1051 | 1058 | 1044 | 1049 | 1078 | 1119 |
| Beltsville | Maryland | 962 | 928 | 924 | 922 | 925 | 935 | 955 |
| Lamberton | Minnesota | 1117 | 1147 | 1164 | 1146 | 1146 | 1207 | 1334 |
| Roseau | | 1316 | 1401 | 1414 | 1400 | 1399 | 1462 | 1570 |
| Columbia | Missouri | 939 | 959 | 966 | 956 | 955 | 993 | 1068 |
| Novelty | | 1439 | 1471 | 1478 | 1466 | 1465 | 1545 | 1663 |
| Holly springs | Mississippi | 1294 | 1301 | 1320 | 1292 | 1308 | 1330 | 1324 |
| Starkville | | 1050 | 1048 | 1054 | 1041 | 1051 | 1070 | 1075 |
| Clayton | North Carolina | 939 | 943 | 947 | 936 | 944 | 948 | 957 |
| Fletcher | | 1186 | 1206 | 1224 | 1202 | 1206 | 1271 | 1369 |
| Mills river | | 1720 | 1783 | 1801 | 1773 | 1788 | 1848 | 1928 |
| Oxford | | 925 | 936 | 936 | 928 | 936 | 956 | 951 |
| Raleigh | | 756 | 776 | 784 | 768 | 777 | 793 | 776 |
| Wallace | | 1493 | 1493 | 1498 | 1480 | 1494 | 1493 | 1438 |
| Williamsdale | | 1493 | 1493 | 1498 | 1480 | 1494 | 1493 | 1438 |
| Lincoln | Nebraska | 2558 | 2603 | 2605 | 2599 | 2598 | 2655 | 2640 |
| Sidney | | 2089 | 2138 | 2147 | 2148 | 2142 | 2168 | 1899 |
| Centerton | New Jersey | 1067 | 1059 | 1060 | 1049 | 1058 | 1080 | 1120 |
| Pittstown | | 1616 | 1661 | 1677 | 1651 | 1670 | 1686 | 1687 |
| Woodstown | | 2573 | 2593 | 2602 | 2580 | 2591 | 2625 | 2659 |
| Clovis | New Mexico | 1756 | 1521 | 1489 | 1552 | 1564 | 1291 | 850 |
| Farmington | | 404 | 356 | 417 | 373 | 377 | 453 | 320 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Custar | Ohio | 2449 | 2450 | 2456 | 2430 | 2447 | 2484 | 2474 |
| Fremont | | 1880 | 1904 | 1918 | 1894 | 1905 | 1947 | 1955 |
| Chickasha | Oklahoma | 1468 | 1510 | 1515 | 1503 | 1501 | 1556 | 1594 |
| Enid | | 980 | 1012 | 1009 | 1006 | 1005 | 1013 | 1017 |
| Fort cobb | | 2532 | 2472 | 2473 | 2477 | 2477 | 2392 | 1487 |
| Goodwell | | 2082 | 1950 | 1984 | 1998 | 2010 | 1545 | 1200 |
| Lahoma | | 1281 | 1338 | 1336 | 1333 | 1331 | 1375 | 1439 |
| Perkins | | 770 | 768 | 764 | 764 | 763 | 762 | 738 |
| Tipton | | 2249 | 2264 | 2268 | 2268 | 2268 | 2252 | 1525 |
| Weatherford | | 1040 | 1058 | 1054 | 1053 | 1052 | 1051 | 1036 |
| State college | Pennsylvania | 2230 | 2281 | 2289 | 2276 | 2279 | 2328 | 2394 |
| Nashville | Tennessee | 1473 | 1495 | 1503 | 1487 | 1500 | 1548 | 1576 |
| Springfield | | 1880 | 1951 | 1988 | 1933 | 1965 | 2046 | 2056 |
| Spring hill | | 1880 | 1951 | 1988 | 1933 | 1965 | 2046 | 2056 |
| Amarillo | Texas | 1827 | 1721 | 1746 | 1728 | 1730 | 1829 | 1379 |
| Bushland | | 1161 | 1238 | 1240 | 1237 | 1238 | 1315 | 1237 |
| Chillicothe | | 1519 | 1504 | 1495 | 1502 | 1501 | 1456 | 1068 |
| College | | 983 | 1000 | 994 | 994 | 993 | 998 | 776 |
| Orange | Virginia | 1831 | 1848 | 1867 | 1842 | 1855 | 1900 | 1948 |
| Petersburg | | 1032 | 1026 | 1038 | 1015 | 1031 | 1052 | 1052 |
| Suffolk | | 899 | 901 | 910 | 894 | 902 | 924 | 923 |
| Alburgh | Vermont | 1339 | 1386 | 1406 | 1377 | 1392 | 1435 | 1547 |
| Torrington | Wyoming | 1953 | 1963 | 1961 | 1966 | 1968 | 1938 | 1581 |