Evaluation of Yield and Agronomic Performance of Leafed and Semi-leafless Pea Blends

A Thesis Submitted to the College of Graduate Studies and Research
In Partial Fulfillment of the Requirements
For the Degree of Master of Science
In the Department of Plant Sciences
University of Saskatchewan

By
Yanben Shen

PERMISSION TO USE

ABSTRACT

Field pea has two distinct leaf morphologies: leafed (L) and semi-leafless (SL). The leafed pea is more susceptible to lodging, but it may have high yield potential when lodging is prevented. In contrast, the semi-leafless pea is bred for lodging resistance and has greater yield stability. However, it has reduced leaf area which may be related to yield potential. When grown together, the semi-leafless type improves the lodging resistance of leafed pea and also gains a weed competition advantage with the leafed blend. There is some evidence that growing blends of leafed and semi-leafless pea may lead to higher crop yield than sole crops of either leaf type. However, previous studies used two different semi-leafless and leafed varieties to compose the leaf-type blend, which identified confounding factors. The effect of pea leaf blends has not yet been clarified, whether the agronomic and yield improvements were from the blends and/or the mixture of different genotypes. An optimum blending ratio has also not been determined. The objective of this thesis was to determine the yield and agronomic performance of near-isogenic pairs of leafed and semi-leafless pea varieties. Two field studies were conducted in Saskatchewan, Canada, from 2017 to 2019. The pea leaf blends reduced leafed pea lodging and prevented leafed pea yield loss compared to the leafed monoculture; however, the blend did not affect light interception, disease, lodging, and yield relative to the semi-leafless monoculture. The estimated optimal blending ratio, 86:14% SL/L, had a 13% yield increase compared to the leafed monoculture, but no yield and agronomic advantage compared with the semi-leafless monoculture. When comparing the pea leaf blends that consisted of the near-isogenic pairs or non-isogenic pairs, light interception, lodging, disease severity, and yield responses of the variety mixtures were not significant. The results of the relative yield in variety mixture to monoculture showed that the blends' yield was generally intermediate to the mean of the two varieties in the monocultures, and no consistent agronomic improvements or yield increases could be attributed to the variety mixture. To explore the effect of leaf type blend on lodging, crop canopy was captured pre-and post- lodging by UAV-imagining. The canopy height was determined using canopy reconstruction of a time-series reduction from UAV-image analysis, which was compared to the lodging index from the ground measures. The image-derived lodging index was more precise than ground reference measures of lodging, and the pure semi-leafless pea was the most lodging resistant ratio. Lodging increased significantly when the leafed percentage in the blend exceeded 33%. This thesis project grew four pairs of

near-isogenic lines in the two experiments at eleven site years and did not find significant yield and agronomic benefits to the current semi-leafless pea production.

ACKNOWLEDGEMENTS

DEDICATION

I dedicate this thesis to myself for sedulous study and everyone helped me at the University of Saskatchewan from 2014 to 2021.

TABLE OF CONTENTS

List of Tables

List of Figures

List of Abbreviations

L	Leafed Pea
SL	Semi-leafless Pea
NIL	Near-isogenic line
LER	Land Equivalent Ratio
AF	Dominant AFILA allele
af	Recessive afila allele
UAV	Unpiloted Aerial Vehicle
SAR	Synthetic Aperture Radar
PAR	Photosynthetically Active Radiation
G	Genotype
GDD	Growing Degree Days
RMSE	Root Mean Square Error
QTL	Quantitative Trait Locus
RYld	Mixture Yields Relative to the Monoculture
RDis	Mixture Disease Relative to the Monoculture
ANOVA	Analysis of Variance
LSD	Least Significant Difference
CDC	Crop Development Center
CV	Coefficient of Variance
DEM	Digital Elevation Map
CHM	Canopy Height Model
DSM	Digital Surface Models
DTM	Digital Terrain Models
GCP	Ground Control Point
RTK	Real-Time Kinetic
NDVI	Normalized Difference Vegetation Index
MGSAVI	Modified-Green Soil-Adjusted Vegetation Index
SAVI	Soil-Adjusted Vegetation Index

1. Introduction

Field pea (*Pisum sativum*) is an important crop in Western Canada. Saskatchewan remains the Canadian pea production leader and exported 2.5 million tonnes of field pea in 2020 (Statistics Canada, 2020). Besides the high economic returns, field pea is a popular rotation crop. Pea is adapted to the cool temperate zone and short growing season in Western Canada. For the farmers, pea reduces the requirement for nitrogen fertilization by forming a symbiosis with rhizobia to fix nitrogen. It also increases crop diversity to help break pest cycles.

Nearly all pea varieties grown in Saskatchewan are now semi-leafless (SL), except for some leafed forage pea (L). The semi-leafless type was bred from the leafed type, and semi-leafless tendrils replace the leaflets (Goldenberg, 1965; Kujala, 1953). The tendrils in the semi-leafless canopy are intertwining, which significantly improves lodging resistance (Stelling, 1989). Enhanced lodging resistance results in decreased disease severity (Banniza et al., 2011) and increased pea yield (Mikic et al., 2011). However, the reduced leaf area in semi-leafless pea can cause reduced weed competition (Wall et al., 1991) and light interception (Heath & Hebblethwaite, 1985). In comparison, leafed leaves are the wild type, and the leaf consists of stipules, leaflets, and a few tendrils. Leafed pea varieties are no longer grown for food uses in Western Canada because compared to semi-leafless varieties they have greater lodging and reduced yield stability (Syrovy, 2014). However, leafed pea had a higher yield potential than semi-leafless pea when lodging is reduced (Zivanov et al., 2018; Gollner et al., 2019).

Blends of the two pea leaf types may complement each other. Blends of leafed and semi-leafless varieties in previous studies had greater yield, lodging resistance, and land-use efficiency than the sole variety (Schouls & Langelaane, 1994; Cupina et al., 2010; Syrovy et al., 2015; Antanasovic et al., 2011;). Schouls and Langelaane (1994) found that a pea leaf blend delayed leafed lodging, which resulted in a higher yield than the sole semi-leafless and leafed peas. Pea leaf blends had an increased land equivalent ratio compared with pure stands in forage production (Antanasovic et al., 2011; Živanov et al., 2018). In organic cropping systems, the blend was found to reduce a

significant amount of weed biomass, and provided an 18% and 156% yield increase to semi-leafless and leafed monoculture, respectively (Syrovy et al., 2015).

The improvement of pea leaf blends is contingent upon the blending ratio of semi-leafless and leafed peas in the mixture. A higher semi-leafless percentage in the blend prevented lodging in a study by Syrovy et al. (2015). In a weed-free study, Schouls and Langelaane (1994) estimated the optimal blend was 53%~67% semi-leafless with 47%~33% leafed. In comparison, Antanasovic et al. (2011) found that a 75:25 ratio resulted in the highest Land Equivalent Ratio (LER), which provided a 9% and 39% yield increase compared to a pure semi-leafless and leafed stand, respectively. However, the leaf-type blend did not ensure a yield benefit compared with sole leaf monoculture. In a study by Gollner et al. (2019), the pure leafed stand produced the highest yield, followed by the 50% semi-leafless and 50% leafed intercrops, and then the pure semi-leafless stand.

Previous studies have shown that blending semi-leafless and leafed pea varieties can improve agronomic and yield performance compared to the sole leaf monoculture. However, these leaf-type blends used different varieties for the leafed and semi-leafless components which could affect the interpretation of these improvements, where variety could be a confounding effect. Darras et al. (2014) studied the semi-leafless variety mixture and found the mixture improved the competitive ability of field pea compared to a pure stand. Schoul and Langelaane (1994) and Zivanov et al. (2018) tested pea leaf blends in the same mixing ratio but found different magnitudes of yield increase. The confounding effects of using separate varieties in blends impacted the blending yield and agronomic performance. Therefore, the effect of pea leaf blends has not yet been clarified, whether the agronomic and yield improvements were from the blends and/or the mixture of different genotypes. An optimum blending ratio has not been determined.

Near-isogenic lines (NILs) have been used in numerous studies to determine alternative gene effects on phenology (Lanning et al., 2012), yield components (Arisnabarreta & Miralles, 2008), biotic stress (Jones et al., 2011) and abiotic stress (Venuprasad et al., 2011). Near-isogenic lines are inbred lines that genetically resemble the recurrent parent, except for a specific genetic locus (Kooke & Wijnker, 2012). Harvey (1972) evaluated the photosynthetic and respiratory net CO_2 exchange of fruits among the

near-isogenic normal and mutant pea types: leafed, vestigial stipule, semi-leafless, and leafless. The study showed that the semi-leafless tendril tissues is 18% CO_2 photoassimilation per unit dry weight than the leafed leaflets. Within the dry weight of leaf tissue, the semi-leafless tendrils were higher than the leafed leaves and the compensation resulted in the photoassimilation performance being similar between semi-leafless and leafed types at the saturated light condition (Harvey, 1972).

Semi-leafless (*af*) and leafed (*AF*) alleles at the *afila* locus control leaflet development. Field pea breeder Tom Warkentin (Crop Development Center, University of Saskatchewan) with assistance from Devini DeSilva, developed leafed-type near-isogenic lines by repeated backcrossing. An *AF* allele, donated by a leafed line, was introgressed into the recipient semi-leafless line, and the new inbred lines were repeatedly backcrossed with selection for the leafed phenotype. After five generations of backcrossing, the leafed NILs genetically resembled the semi-leafless parental line. Mixing the leafed NIL with its semi-leafless parental line could clarify the potential benefits of pea leaf blends and establish a trustable blending ratio by excluding the confounding effects caused by mixing non-isogenic varieties.

In this thesis, it was hypothesized that the mixture of leafed and semi-leafless peas would improve yield and agronomic performance when compared with the sole leaf type. The two experiments evaluated the leafed NIL and semi-leafless pairs developed in four genetic backgrounds to address three objectives:

1. To compare the near-isogenic blends with the same varieties grown as leafed or semi-leafless monocultures;

2. To detect whether intercepted solar radiation, disease-resistance, lodging performance, biomass, and yield varied by growing a varietal mixture;

3. To determine an optimal ratio of NIL pairs that have low disease, reduced lodging, and optimal yield across four genetic backgrounds.

At the conclusion of this thesis, the study will establish a comprehensive statement for the semi-leafless and leafed blend.

2. Literature Review

2.1 Pea Production

Canada is the world field pea production and export leader, with a production of 4.6 million tonnes in 2020 (Statistics Canada, 2021). As an important food, field pea contains high levels of nutrients, including carbohydrates, protein, and minerals along with low fat and sodium (USDA Nutrient Database, 2018). Typically, pea is used for human consumption and animal feed. Strong demand from India, China, and the United States has resulted in a consistent market for Canadian dry peas. Of the 4.6 million exported tonnes in 2020, Saskatchewan accounted for 54.4% of production or 2.5 million tonnes. Research conducted at seven different locations in Saskatchewan showed that pea production is suited to most agro-ecological zones in Saskatchewan (Government of Saskatchewan, 2017). As a cash crop, the price of dry pea reached approximately $250 tonne^{-1} in 2017 and $365 tonne^{-1} in 2015 (Government of Saskatchewan, 2018). Besides significant profit potential, field pea is well suited for rotation with canola and wheat for nitrogen fixation, interruption of diseases and insect life cycles, microbial biodiversity and many other sustainable benefits (Saskatchewan Pulse Grower, 2017).

2.2 Pea growth and physiology

Field pea is a diploid, annual, herbaceous plant with a raceme inflorescence. Pea is well-adapted to cool spring seeding conditions and starts flowering approximately 40-55 days after seeding under long day photoperiods. The optimum growing temperature is between 13 to 18°C and crop maturity is achieved about 80-110 days after seeding (Maiti et al., 2012). The favorable soils for pea production are well-drained clay loam soils. In Western Canada and the Northern United States, field pea is usually seeded in mid-April to early May, to utilize snow melt and target harvest before fall frosts. Despite a shallow root system, field pea is relatively adapted to drought stress; however, low soil moisture during the flowering and podding stages can significantly reduce harvest yield (Bueckert et al., 2015).

2.3 Agronomic factors affecting pea production

2.3.1 Weeds

Weed competition is a severe problem in field pea. Weeds suppress pea development and decrease yields, particularly at the early stages. Harker et al. (2001) studied pea competition with wild oat (*Avena fatua*) and tartary buckwheat (*Fagopyrum tataricum*), with the field pea seeded in 23-cm rows, at a rate of 211 kg ha^{-1} and a depth of 5 cm. They found that the critical weed-free period for this crop was 1 to 2 weeks after pea emergence, and weeds that were present for the full-season decreased yield by 40 to 70% (Harker et al., 2001).

2.3.2 Pests and diseases

Diseases reduce yield and impact seed quality in field pea. Most of the field pea diseases are caused by fungal pathogens. Ascochyta blight is one of the most common pea diseases globally. In Western Canada, other field pea diseases include seedling blight, root rot, and powdery mildew; however, recently registered pea varieties are resistant to powdery mildew (Saskatchewan Variety Guide, 2021).

2.3.3 Lodging

Lodging is a major agronomic problem in plant growth, where the stem bends near the soil surface. This may cause the whole plant to lie flat on the ground at harvest. Lodged canopies cause a warm and humid micro-environment that increases disease infections (Wang, 1998). Setter et al. (1997) found lodging decreased the light penetration for photo-assimilation in leaves. For example, when comparing a lodged and non-lodged plant canopy, light was intercepted within the top 5 cm for lodged and top 80 cm for non-lodged. Lodging also reduces canopy photosynthesis and leads to yield loss (Setter et al., 1997). Amelin and Parakhin (2003) found that severe lodging reduced yield by 74%. Lodging constrains combine harvest, with Schouls and Langelaan (1994) reporting that machine harvested lodged peas were 30% lower yielding than hand harvested peas.

2.3.3.1 Factors causing lodging

The field pea stem has a weak base, and pod filling on the shoots leads to higher lodging risk mid-way and late in the season. Smitchger (2017) demonstrated that pea lodging was related to stem diameter, plant height, tendril length, stem brittleness, and plant

branching. Smitchger et al. (2020) found that plant height is strongly correlated with lodging susceptibility, whereas stem diameter is negatively correlated. Beeck et al. (2006) found lodging resistance strongly correlates with compressed stem thickness but only weakly associates with stem diameter. Bilgili et al. (2010) determined that taller, determinate peas with heavier pod filling could be more prone to lodging. Singh and Srivastava (2015) measured the effect of plant height, stem diameter, and the internode length of the first bend on lodging and yield in normal leafed, semi-leafless, and acacia leaf types. The results showed that these traits had no significant correlation with lodging severity, however, the semi-leafless type consisting of intensively twisting tendrils had significantly lower lodging. In contrast, Smitchger et al (2020) stated that some varieties with semi-leafless leaf types also had severe lodging. Lodging is also sensitive to environmental effects, such as rain, hail, and wind. Although no wind study has been conducted on pea for lodging, a wind lodging study in corn indicated that wind shear reduced yields by 2-6%, 5-15%, and 13-31% when corn was at early (V10-V12), medium (V13-V15), and late (V17-R1) growth stages, respectively (Carter & Hudelson, 1988).

2.3.3.2. Measuring lodging

Lodging is usually measured in the field by visual rating. Lodging score is commonly used to describe pea lodging at the flowering and ripening stage, using predefined numerical scales with endpoints denoting no to severe lodging (Stelling, 1989; Bilgili et al., 2010). Stelling (1989) used a visual lodging score, crop height, and lodging angle to evaluate pea stability from flowering to harvest. The study concluded that the visual score measure was the most suitable method of assessment, as it saves time and has acceptable precision; however, it may overestimate the score at the harvest stage if only part of the stem is lying flat on the ground. Lodging score in pea breeding and agronomic trials in North America and Europe over the past three decades typically use a 1-9 scale where 1=no lodging to 9=completely lodged. This scale has proven to be highly effective and reproducible (Warkentin, personal communication). Remote sensing provides an automated method to measure lodging severity instead of relying on human labor in the field. In comparison with non-lodged plants, the lodged plants have different reflected spectrum wavelengths caused by changes related to plant geometry (leaf area, distribution of leaves and stems), crop morphology (height and

biomass), and biochemical content such as chlorophyll (Hosoi & Omasa, 2012; Holman et al., 2016; Murakami et al., 2012).

Remote sensing studies can be grouped into three categories based on the platforms used: ground-based, drone-based, and space-based. The ground-based platform has less noise and high resolution, and it observes plant stems underneath the canopy. Liu et al. (2012) used hyperspectral sensors to capture the wavelength from 300 nm to 2500 nm, and the resulting image analysis classified the non-lodged and lodged rice crop with a 97.8% overall accuracy.

The use of Unoccupied Aerial Vehicle (UAV) has increased for remote sensing use in recent years due to the spatial resolution, high throughput, and acceptable price. Several study have used UAVs carrying multispectral and LiDAR sensors to model the point cloud of crop canopy that can detect crop plant height (Fumiki, et al., 2012). Hu et al. (2021) used UAV-LiDAR to collect different growth stages of maize to obtain lodging severity, with an accuracy of the image estimation of $R^2 = 0.9824$, root mean square error (RMSE) = 0.0613 m.

Satellite networks offer worldwide geographic observation with diverse remote sensor accessibility for precision agriculture. Synthetic Aperture Radar (SAR) sensors can penetrate the atmosphere and plant canopy, avoiding the noise of clouds and providing a reliable repeated image collection. The ratio between the root anchorage strength and the self-weight moment of the whole plant was estimated from the satellite SAR images to determine root lodging susceptibility in wheat (Chauhan et al., 2021).

2.4 Pea leaf type

2.4.1 Leaf Type

There are two major morphological leaf type classes in commercially grown field pea: semi-leafless and leafed types. Leafed (conventional; *AFAF TLTL*) pea has a compound pinnate leaf arrangement and consists of a basal stipule, leaflets, and crown tendril structures (Lafond et al., 1981). The innovation in pea leaf morphology was the breeding of a new leaf type, a semi-leafless type (*afaf TLTL*), which consists of basal stipules with intensive tendrils (Goldenberg, 1965; Kujala, 1953). The restructured leaf type was due to mutations of the qualitative alleles, the AFILA (*AF*) and TENDRIL-LESS (*TL*), that influences the presence or absence of the leaflet and tendril structures

(Kujala, 1953; Goldenberg 1965; Hagem, et al., 1911; Villani and Demason, 1997). Kujala (1953) and Goldenberg (1965) first discovered the *af* gene that resulted in the semi-leafless type. The recessive *af* allele led to the phenotype where compound tendrils occurred in proximal positions and simple tendrils occurred in the distal position at the lateral primordium development stage (Kujala, 1953). The *TL* gene was first discovered by Hagem, et al. in 1911. As the name suggests, the *TL* gene regulates the leaflet primordia in leaf development and only tendrils grow (Gourlay et al., 2000). The recessive *tl* allele led to the occurrence of laminar leaflets instead of normal tendrils, and it was named the *acacia* phenotype (Hagem, et al., 1911).
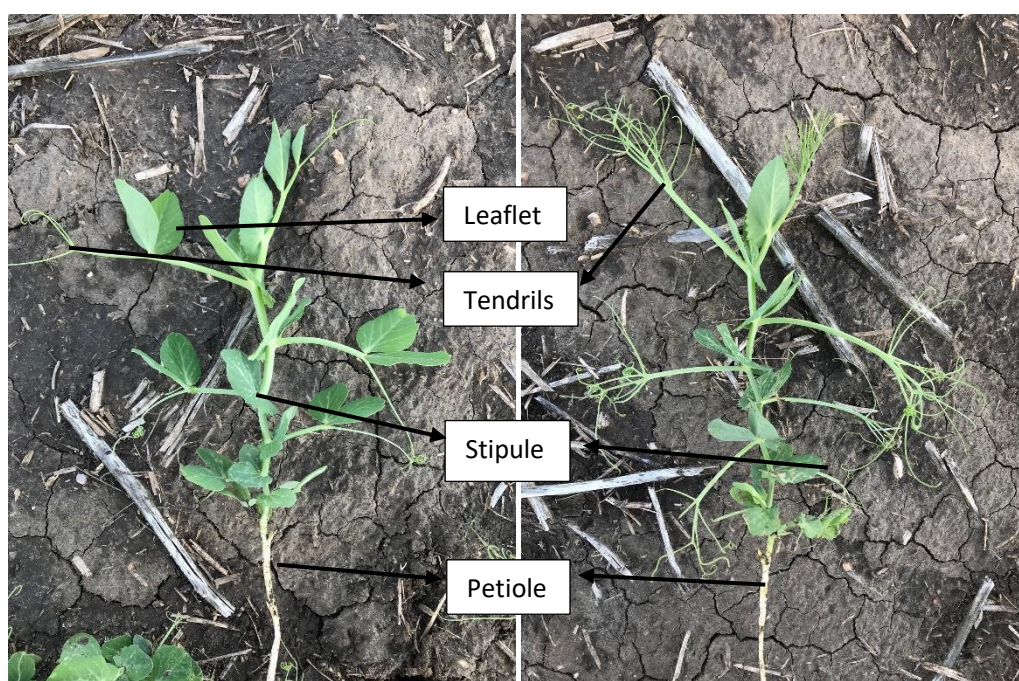
Figure 2.1 Aboveground leafed plant (left) and semi-leafless plant (right).

2.4.2 Normal Leafed Pea

Normal leafed pea varieties are generally grown for forage in Western Canada (Saskatchewan Variety Guide, 2021). Research on pea-leaf light interception found that leafed pea intercepted more solar radiation than semi-leafless pea in moisture-stressed conditions (Heath & Hebblethwaite, 1985). Gollner et al. (2019) reported that leafed pea developed higher leaf area index and nitrogen fixation than semi-leafless pea, resulting in greater photosynthesis. Furthermore, Spies, et al. (2010) determined that vine length and the leafy characteristic were related to weed competitiveness. Plots with leafed field pea varieties had reduced weed biomass and yield loss than semi-leafless varieties in weedy conditions (Harker et al., 2008).

Leafed peas are peculiarly prone to lodging, which has caused leafed pea production to dwindle. A study in northern Europe found that natural lodging frequently occurred in leafed pea and resulted in approximately 45% seed yield loss (Schouls & Langelaan, 1994). Živanov, et al. (2018), Mikic et al. (2011), and Gollner et al. (2019) found leafed pea types have the potential to produce high yields if lodging is prevented. To prevent lodging, several studies were conducted on intercropping leafed pea with supporting plants. Podgórska-Lesiak and Sobkowicz (2013) found lodging was reduced when leafed pea was cultivated with a stiff erect crop (i.e., barley, oat, wheat). Lodging tolerance and yield of leafed pea may also be improved by intercropping with triticale (Gronle et al., 2015) and flax (Klimek-Kopyra et al., 2015); however, in some cereal-pea intercrops, the cereals were found to suppress pea growth (Jensen, 1996).

2.4.3 Semi-leafless Type

Semi-leafless pea varieties with intensive intertwining of extra tendrils tend to lodge later and at a slower lodging rate, which makes the semi-leafless pea ameliorate more lodging resistant compared to leafed pea (Stelling, 1989; Bilgili et al., 2010; Agriculture and Agri-Food Canada, 2012). In addition, the absence of leaflets on the semi-leafless type led to a cooling effect in the canopy microclimate, which inhibited pathogen development (LeMay et al., 2009). Banniza et al. (2011) found that Mycosphaerella blight severity was significantly lower in semi-leafless than in leafed pea, and the severity was positively correlated to lodging tendency. Greater lodging and lower disease susceptibility allows semi-leafless pea to produce greater yield and have higher yield stability than leafed pea under normal growing conditions (Mikic et al., 2011).

2.5 Blending Research

Leafed and semi-leafless pea types, when grown together may have complementary field performance in leaf area, lodging, diseases, and weed competition. When growing two leaf types simultaneously, the semi-leafless type could improve lodging resistance of leafed pea, and the leafed type could simultaneously provide weed competition and photosynthetic advantages for the semi-leafless type. Leaf area, solar radiation interception, and solar use efficiency are among the main determinants of crop

productivity (Yahuza, 2011; Lecoeur & Ney, 2003; Kof et al., 2004). The leaf-type blends may alter canopy leaf orientation and composition, resulting in greater overall canopy area than sole varieties, and allow solar radiation to be intercepted and used more efficiently. Thus, the blending of leaf-types may lead to higher crop yield than the sole crops of either leaf type. Intercropping leafed and semi-leafless pea has shown delayed lodging and increased seed yield by 5-11% compared to sole types (Schouls & Landelaan, 1994). Cupina et al. (2010) found that a leaf blend produced 100 - 200 kg ha^{-1} higher seed yield than the sole leaf types.

Research at the University of Saskatchewan indicated that blending semi-leafless with leafed pea increased yield under organic cropping systems. Syrovy et al. (2015) mixed leafed and semi-leafless varieties in a 25:75% ratio and found that seed yield of the mixture was 12% higher than the semi-leafless monoculture. The yield advantage occured due to the leafed variety in the mixture yielding nearly threefold greater than in the monoculture. An additional experiment conducted under organic systems at the University of Saskatchewan testing different varieties indicated that 75:25% SL/L mixtures comprised of either 40-10 (leafed), Trapper (leafed), or CDC Sonata (leafed) paired with CDC Meadow (semi-leafless) had a 13 and 21% higher yield than the respective CDC Meadow and leafed varieties alone (Syrovy, 2014). In contrast, Gollner et al. (2019) reported no yield increase with a 50:50% mixing ratio in an organic system.

2.5.1 Blending Ratio Research

Blending ratio is a critical factor for a leaf-type blend and deciding which type is the dominant component, thus, affecting lodging tolerance and disease development. The yield of leaf-type blends in previous studies surmised that the optimum blend has a semi-leafless proportion of 50% or more (Schouls & Landelaan, 1994; Cupine et al., 2011; Syrovy et al., 2015). Syrovy (2014) demonstrated that lodging resistance of leaf-type blends increase as the percentage of semi-leafless pea increases and recommended a minimum of close to 50% semi-leafless pea as a starting point for the ratio. An optimal seeding ratio has not been established however, since previous studies used different varieties to compose the leaf-type blend. This could present confounding factors and influence the optimum blending ratio, yield, and agronomic performance of the leaf blend.

2.6 The effect of varietal mixtures in disease resistance, yield stability, and yield

Varietal mixtures use the intra-specific effects of two varieties to enhance the diversity, with relatively easy management and low cost for farmers. The advantages of varietal mixture over their monoculture have been found in various agronomic aspects including: increasing the range of disease resistance to break pathogen expansion (Finckh, et al., 2000), stabilizing yield under environment stress (Henry et al., 2016), and increasing harvest yield (Smithson & Lenne, 1996). Varietal mixtures reduce air-borne disease epidemics by decreasing the spatial density of the susceptible plants in the canopy, providing a barrier of less susceptible plants for disease spread, and increasing the genetic diversity in a given environment (Vallavieille-Pope, 2004). In a study using a varietal mixture of 25% resistant/75% susceptible to control *Septoria tritici* blotch in durum wheats, the mixture suppressed disease severity by 48% compared to the susceptible pure stands (Sarrah et al., 2019). The effects of host genotypes on disease appears to be variable and environmentally influenced (Cowger & Mundt, 2007).

Different varieties in a mixture could compensate and facilitate each other when plants confront pests and lodging conditions; thus, yield stability of varietal mixtures can be generally greater than variety monoculture (Smithson & Lenne, 1996). In barley, the yield of varietal mixtures was as high as the best performing monoculture, and the yield stability of the mixture was better than the monocultures (Creissen, et al., 2016).

The effect of variety mixture on yield is not well understood since it varies quantitatively for genotypes and environment. The previous review papers on varietal mixtures state that the yield improvement by the mixtures appear to be inconsistent and not guaranteed (Smithson and Lenne, 1996; Freville, et al., 2019). Simmonds (1962), Trenbath (1974), and Jensen (1996) found that mixture yields usually lie above the mean of the relative components in the monoculture. A two-year field research project showed that varietal mixtures had similar yield and biomass compared with the monoculture in a year with favorable growing conditions but in the following drought year, the mixture was found to have improved water use efficiency and yield compared with the monoculture (Qin, et al .2019). By looking at the meta-analysis of hundreds of studies over many decades, their results showed that varietal mixtures had a higher average yield than the variety pure stands by 2~3% (Kristoffersen, et al., 2020; Kiaer et al., 2009). Integrating the short-term and long-term studies, authors illustrated that

the variety mixtures have greater stability than the monoculture in stressed environments, which enhances long-term profitability.

2.6.1 Utilizing near-isogenic blends to determine the effect of varietal mixture

Previous studies of pea leaf blends using the leafed and semi-leafless components were from distinct genetic backgrounds (Schoul & Landgelaan, 1994; Syrovy et al., 2015; Gollner et al., 2019). Different varieties were the confounding factor for effectively determining the influence of leaf type on yield, disease, and weed pressure and prevented the development of a dependable blending ratio. The yield increase of leaf-type blends compared to leaf-type monoculture varied quantitatively in previous studies, due to the interaction of the genotype and leaf-type effects (Schoul & Landgelaan, 1994; Syrovy, 2014). Forcella (1987) stated that comparing near-isogenic lines (NIL), that differ only in the trait of interest, is the only conclusive way to determine a difference in response to a specific characteristic.

The NIL is usually generated via a repeated backcrossing method. The donor parent, having a genetic segment of interest, is crossed with the recurrent parent variety (usually a reliable variety) to introgress the segment (Blanco, et al., 2006; Brouwer, et al., 2004). The progenies inherited from the introgression are then selected for the interested trait and continually crossed with the recurrent parent. Resulting lines are called near isogenic lines. After five to six generations, the selected progenies are nearly identical to the recurrent parent, except the introgressed gene resulting in a NIL (Stam & Zeven, 1981).

NILs have been extensively used in plant sciences to study pathology, physiology, and phenology. Hamard et al. (2021) used NILs carrying a single or combinations of resistant quantitative trait loci (QTLs) to determine their ability to resist Aphanomyces root rot (*Aphanomyces euteiches*) infestation in French populations of field pea. Previous studies have measured the effect of photosynthetic and respiratory net CO_2 exchange on pods of the NILs of semi-leafless, leafed and leafless leaf types (Harvey, 1978; Harvey & Goodwin, 1978).

3. Chapter 1: Utilizing near-isogenic lines to determine yield and agronomic advantages of leafed and semi-leafless field pea blends

Abstract

Field pea (*Pisum sativum*) has two distinct leaf morphologies: leafed (L) and semi-leafless (SL). Grown together, semi-leafless and leafed pea blends have shown better lodging resistance than leafed pea monoculture with greater weed suppression, solar-radiation interception relative to the semi-leafless pea monoculture, and further led to higher crop yield than sole crops of either leaf type. Previous studies have only investigated mixing leaf types from two distinct genotypes, and therefore the blend could be affected by traits specific to each pea variety rather than the difference in leaf type. To determine if yield and agronomic improvement of pea leaf blends are due solely to leaf type, this study, for the first time: 1) compared leaf blends of near-isogenic lines with the same varieties grown in monoculture, and 2) determined whether intercepted solar radiation, disease-resistance, lodging performance, biomass, and yield were improved by growing a varietal mixture. Five field experiments tested all possible pairings of four near-isogenic leafed and semi-leafless lines in a single ratio (75:25 SL/L), compared to monocultures (100% SL or 100% L). The results showed near-isogenic blends of semi-leafless and leafed pea decreased lodging by 11% compared to the leafed monoculture. The semi-leafless monoculture had a 13.4% greater yield compared to the leafed monoculture, while the blends showed statistically similar yields compared to the leafed monoculture. The near-isogenic blends and non-isogenic blends had no significant difference on disease severity, yield, and yield stability. The study determined that the main effect of using pea leaf blends was preventing yield loss due to lodging in the leafed component, but this did not increase the seed yield of the semi-leafless component. Consequently, the exhaustive comparison of near-isogenic or non-isogenic blends grown under multiple environments indicates that semi-leafless and leafed pea blends have non-significant yield and agronomic advantages compared to semi-leafless monocultures.

3.1 Introduction

Field pea (*Pisum sativum*) has two distinct leaf morphologies: a leafed (L) type, with

compound pinnate leaves extending from the stipule, and semi-leafless (SL), with the leaflets having been bred into tendrils. The development of semi-leafless pea types has led to higher resistance to lodging, caused by the stem bending near the soil surface. The whole plant may lie flat on the ground at harvest (Mikic et al., 2011; Bilgili et al., 2010; Uzun & Açikgöz, 1998; Heath & Hebblethwaite, 1984). The absence of leaflets in semi-leafless pea varieties reduces weed competition (Harker et al., 2008; Munakamwe et al., 2012). Previous research indicates that leafed and semi-leafless pea blends are complementary to each other. When grown simultaneously, the semi-leafless type improve leafed lodging resistance (Schouls & Langelaan, 1994). Leafed and semi-leafless pea blends can also have a weed competitive advantage relative to the semi-leafless monoculture (Syrovy et al., 2015). Furthermore, leafed and semi-leafless pea blends may have an altered canopy leaf orientation and composition, with leafed leaflets filling the canopy space left open by the semi-leafless tendrils (Antanasovic et al., 2011). The leaf-type blend will result in a greater overall canopy area than the sole varieties, allowing solar radiation to be intercepted and used more efficiently. Since the interception and efficiency of solar radiation are among the main determinants of crop productivity (Yahuza, 2011; Lecoeur & Ney, 2003), growing blends of leafed and semi-leafless pea may lead to higher crop yield than the sole crops of either leaf type.

Previous research has shown this (Schouls & Langelaan, 1994; Syrovy et al., 2015; Cupina et al., 2010). The mixture of two leaf types delayed lodging and seed yield was 5-11% higher than the sole types (Schouls & Landelaan, 1994).. Syrovy et al. (2015) observed a 75:25 SL/L blend led to a 18% and 156% yield increase over the semi-leafless and leafed monoculture respectively, under organic cropping systems. However, the benefits of pea leaf blends to leaf monoculture were inconsistent. Gollner et al. (2019) conducted field pea blends in a 50:50 SL/L mixing ratio with varied varieties and reported no important yield increase.

Figure 3.1. Diverse leaf-types: **a** leafed pea monoculture, **b** semi-leafless and leafed pea blend, **c** semi-leafless pea monoculture.

Previous studies mixed two leaf types from two different genotypes, presenting a confounding effect of varietal mixture. The agronomic and yield improvements could be a result of variety rather than leaf type. To definitely determine the yield and performance of the blends, it is important to mitigate this effect. Near-isogenic lines (NIL) are newly bred lines that genetically resemble the recurrent parent, except that a small genetic fragment from a donor parent linked to the desired trait is introgressed into the recurrent parent genome (Stam & Zeven, 1981). The NIL is expected to be homozygous and express most of the same characteristics of the recurrent parents, except for the introgression. Comparing NILs differing in the trait of interest is a conclusive method to test a specific characteristic. Harvey (1987) compared the carbon dioxide photo-assimilation potential of near-isogenic leafed, semi-leafless, and leafless types. The current study compared blends consisting of near-isogenic leafed pea and the related semi-leafless pea of four separate pea genotypes.

The objective of this study was to: 1) compare the near-isogenic blends with the same varieties grown as leafed or semi-leafless monoculture, and 2) detect whether intercepted solar radiation, disease-resistance, lodging performance, biomass, and yield will differ in the near-isogenic and non-isogenic blends.

3.2 Materials and Methods

3.2.1 Pea variety and development of Near Isogenic Lines (NILs)

This project cooperated with the Crop Development Centre (CDC), University of Saskatchewan. In 2017-2018, four high-yielding semi-leafless field pea varieties were used: CDC Dakota (dun-type), CDC Amarillo (yellow cotyledon), CDC Striker (green cotyledon), and CDC Centennial (yellow cotyledon) (Table 3.1).

Table 3.1. Characteristics of field pea varieties[*] tested in field studies (2017-18)

	CDC Amarillo	CDC Striker	CDC Dakota	CDC Centennial
Leaf type	Semi-leafless	Semi-leafles	Semi-leafless	Semi-leafless

Market Class	Yellow	Green	Dun	Yellow
Years tested	10	10	9	2
Yield % of CDC Amarillo	100	81	101	95
Relative Maturity	M	M	M	M
Lodging score[a]	3.5	3.5	3.5	5.5
Vine Length (cm)	85	80	85	68
Mycosphaerella blight score[b]	4.5	4.5	4.5	6.4
Powdery Mildew	R	S	R	R
Seed Weight (g/1000)	230	240	205	260

[*](Saskatchewan Variety Guide, 2021).

[a]Lodging score (1-9) where 1 = completely upright, 9 = completely lodged.

[b]Mycosphaerella blight score (1-9) where 1 = no disease, 9 = completely blighted.

Powdery Mildew where R = resistant, S = susceptible.

The NILs of leafed genotypes were bred by DeSilva and Warkentin (2016) at the Crop Development Centre, University of Saskatchewan. The leafed (*AF*) allele was introgressed into the semi-leafless background of the four varieties (Table 3.1) using CDC Sonata (leafed variety; yellow market class) as the donor for the normal leaf (AF) allele through repeated backcrossing. The *AF* allele controls leaflet development; the leaflets are present in the dominant *AF* allele but absent in the recessive *af* allele. CDC Sonata was crossed with each of the four semi-leafless varieties to introgress the *AF* allele into the semi-leafless line (*afaf* gene). In the F_1 generation, all lines were 100% *AFaf* gene. Offspring having the leafed phenotype were selected and repeatedly crossed with the relevant semi-leafless parent variety. In the F_2 generation, crossing the leafed lines and semi-leafless varieties resulted in the offspring being 25% *AFaf* gene, and 75% *afaf* gene. The leafed phenotypic lines were continuously selected and repeatedly crossed with the relevant semi-leafless parent variety until the *AF* segment remained and other introgressions were lost. After five cycles of backcrossing the harvested leafed progenies were the NILs that genetically resembled the semi-leafless parents. As

a result, the leafed NILs were 96.875% semi-leafless parent and 3.125% CDC Sonata, including the introgressed *AF* allele.

3.2.2 Experimental Design and Management

The study was conducted in 2018 (2 site-years) and 2019 (3 site-years) at the Kernen Crop Research Farm (latitude 52º 09', longitude 106º 32') and the Rural Municipality of Blucher No. 343 (latitude 52º 04', longitude 106º 43') in Saskatoon, Saskatchewan for a total of five site-years. The experimental treatments were arranged in an alpha lattice design with four replications.

The study tested all possible pairings of leafed and semi-leafless lines for CDC Amarillo, CDC Centennial, CDC Dakota, and CDC Striker in a single ratio (75:25 SL/L) and included monocultures (100%; see Table 3.2). The treatments were grouped as four categories: near-isogenic blends, non-isogenic blends, semi-leafless monoculture, and leafed monoculture.

Table 3.2 Near-isogenic and non-isogenic pairs of semi-leafless and leafed lines grown in trials at two locations in 2018 and 2019.

Near-Isogenic Pairs (75% SL : 25% L)	Non-isogenic Pairs (75% SL : 25% L)	Monocultures (100%)
CDC Amarillo (SL) : CDC Amarillo (L)	CDC Amarillo (SL) : CDC Centennial (L)	CDC Amarillo (SL)
CDC Centennial (SL) : CDC Centennial (L)	CDC Amarillo (SL) : CDC Dakota (L)	CDC Amarillo (L)
CDC Dakota (SL) : CDC Dakota (L)	CDC Amarillo (SL) : CDC Striker (L)	CDC Centennial (SL)
CDC Striker (SL) : CDC Striker (L)	CDC Centennial (SL) : CDC Amarillo (L)	CDC Centennial (L)
	CDC Centennial (SL) : CDC Dakota (L)	CDC Dakota (SL)
	CDC Centennial (SL) : CDC Striker (L)	CDC Dakota (L)

	CDC Dakota (SL) : CDC Amarillo (L)	CDC Striker (SL)
	CDC Dakota (SL) : CDC Centennial (L)	CDC Striker (L)
	CDC Dakota (SL) : CDC Striker (L)	
	CDC Striker (SL) : CDC Amarillo (L)	
	CDC Striker (SL) : CDC Centennial (L)	
	CDC Striker (SL) : CDC Dakota (L)	

Blend ratio of 75% semi-leafless and 25% leafed, and monocultures of the same lines grown at 100% semi-leafless or leafed. L = leafed, SL = semi-leafless.

The experiments were located on a clay loam Sutherland soil (Dark Brown Soil Zone). Pea was sown with a hoe-opener seeder, in early May of each year at a target density of 88 plants m^{-2} in 2 x 6m plots, with 6 rows spaced 30-cm apart (Table 3.3). Monoammonium phosphate ($NH_4H_2PO_4$) fertilizer containing 16.5 kg ha^{-1} P_2O_5 and 3.85 kg ha^{-1} N was applied with the seed. TagTeam® granular inoculant (*Penicillium bilaii and Rhizobium leguminosarum), (*Novozymes North America Inc., Franklinton, North Carolina, U.S.A.) was also applied with the seed at 4.6 kg ha^{-1}. Plant counts were done xx days after emergence to ensure uniform crop establishment. An in-crop herbicide, Odyssey® (Imazamox 35% a.e. + Imazethapyr 35% a.e.) (BASF Canada Inc., Mississauga, Ontario, Canada), was used for weed control at the V3 growth stage . Peas were desiccated with Reglone® Ion (Diquat ion 20% a.e.) (Syngenta Canada Inc. Guelph, Ontario, Canada) at the R7 growth stage.

Table 3.3 Dates of planting, harvest, and data collection for 2018-2019 experiments in central Saskatchewan, Canada in 2018 and 2019.

Activity	2018		2019		
	Kernen One	Kernen Two	Kernen One	Kernen Two	Blucher

Seeding	5-May	8-May	7-May	21-May	9-May
Plant counts	28-May	28-May	28-May	6-Jun	10-Jun
Light interception[*]	18 Jun (V6), 2 Jul (R2), 8 Jul (R4)	18 Jun (V7), 2 Jul (R2), 7 Jul (R3)	18 Jun (V6), 28 Jun (V8), 20 Jul (R4)	26 Jun (V6), 9 Jul (V9)	19 Jun (V6), 5 Jul (R2)
Disease severity	20-Jul	14-Jul	22-Jul	-	30-Jul
Lodging measurement	21-Jul	20-Jul	22-Jul	10-Aug	30-Jul
Maturity	24 Jul - 5 Aug	16 Jul - 23 Jul	-	-	-
Biomass Sampling	24-Jul	17-Jul	11-Aug	15-Aug	2-Aug
Harvest	7-Aug	29-Jul	20-Aug	28-Aug	28-Aug

[*]The letters and numbers for the light interception represent the pea growing stages at each measurement.

3.2.3 Data Collection

Lodging height index was collected at the R4 growth stage (pod filling). Five random plants were selected from each plot. The distance from the soil surface to the end of the uppermost tendril was measured and considered to be the plant length. Canopy height measurements were taken at the same time by measuring in five random areas of the plot; however, plants were not straightened. Lodging height index was calculated by dividing canopy height by the entire plant length. This calculation can detect the slanted angle of the canopy to determine the lodging percentage, compared to no lodging using the equation (lodging height index=1) (Stelling, 1989):

Lodging height index= Canopy height / Straight plant length (*Equation 1*)

Disease severity was scored at the R4 growth stage. Five random plants were collected from the entire plot and rated for disease severity using an incremental scale. The incremental scale was a 0-10 rating with a 10% category interval of percent affected. The severity was scored based on the percentage of symptoms shown on the whole

plant (Chiang et al., 2014).

Light interception data was collected weekly from the early vegetative to the reproductive stages (Adams and Arkin. 1977). A LI-191R line Quantum sensor (LI-COR) was used to measure photosynthetically active radiation (PAR; μmol s^{-1} m^{-2}) under the plant canopy. The one-meter line quantum sensor was inserted perpendicular to the crop rows in the center of each plot. Simultaneously, another Quantum sensor captured photosynthetically active radiation (μmol s^{-1} m^{-2}) above the canopy. The two below-canopy readings were averaged for each plot, and the proportion of plant light intercepted was calculated using the equation (Purcell et al., 2002):

Intercepted PAR = 1 – (PAR below canopy / PAR above canopy) (*Equation 2*)

Crop maturity was estimated daily after 50% of the pods on the plant turned yellow to golden-brown. Plots were considered mature when 80% of the pods turned brown in color.

Biomass was harvested from two center crop rows using two 0.25 m^{-2} quadrats placed 50 cm from the plot edge in both the front and back of each plot after pod fill. Plants were cut at the ground level, the semi-leafless and leafed pea samples were then separated and placed in bags. The samples were dried in a 70°C oven for 48 hours and then weighed to obtain dry biomass.

Seed yield of the entire plot was captured with a small plot combine as it was impossible to separate the tangled vines of the edge rows. Seed samples were bagged and dried to a uniform moisture content for 24 hours. After drying, the seed samples were cleaned to remove weed seeds and ashes with a KornServiceTM sieve machine(*Continental Agra, Newston, Kansas, U.S.A.*) and then weighed. The area of calculated seed yield excluded the area sampled for biomass.

Mixture yields relative to the monoculture (RYld) was calculated as:

*RYld = Ym / (Ysl * 0.75 + Yl * 0.25)* (*Equation 3*)

Where *Ym* is the actual yield of the leaf mixture, *Ysl* is the yield of the semi-leafless monoculture, and *Yl* is the yield of the leafed monoculture. The 0.75 and 0.25 is the designed proportion of leafed or semi-leafless pea in the mixture.

A similar approach was utilized to calculate mixture disease relative to the monoculture (RDis).

RDis = Dm / (Dsl * 0.75 + Dl * 0.25) (*Equation 4*)

Where *Dm* is the actual disease severity of the leaf mixture, *Dsl* is the disease severity of semi-leafless monoculture, and *Dl* is the disease severity of leafed monoculture. The 0.75 and 0.25 is the designed proportion of leafed or semi-leafless pea in the mixture.

Yield stability was estimated by ecovalence using Wricke's model (Wricke 1962). The ecovalence measures the contribution of a set of genotypes to the total yield in different environments (Annicchiarico. 2002). The lower Wi indicates higher yield stability. Wricke's model is:

W_i (*ecovalence*) $= \Sigma_{ij} (x_{ij} - \bar{x}_{i.} - \bar{x}_{j.} + \bar{x})^2$ (*Equation 5*)

x_{ij}: yields of genotype *i* in *j* environment
$\bar{x}_{i.}$: mean of yields of genotype *i* across environments
$\bar{x}_{j.}$: mean of yields across genotypes in environment *j*
$\bar{x}$: grand mean

3.2.4 Statistical Analysis

An analysis of variance (ANOVA) was performed using SAS 9.4 (SAS Institute Inc., Cary, North Carolina, U.S.A.). To meet the assumptions of ANOVA, PROC UNIVARIATE and the Levene's test were used to test residual normality and homogeneity of variance, respectively. Where residuals did not meet the assumption, the REPEATED/GROUP statement of PROC MIXED was used to adjust the covariance structure to correct for heterogeneity. Random effects were estimated using

the COVTEST statement of PROC MIXED to determine whether data could be combined over site-years.

A Fisher's protected least significant difference (LSD) was used to detect treatment differences at $p < 0.05$. The REPEATED/SUBJECT statement in PROC MIXED was used to analyze repeated measures (light interception). The best-fit covariance structure to the model was determined by the lowest AICC value.

To account for spatial variability among the plots, a spatial location coordinate was established for all individual plots as north and east. The REPEATED statement specified the structure of the covariance matrix. TYPE specified the spatial covariance EXP (exponential) model to be estimated (Marx and Stroup 1993).

To determine the effect of pea leaf blends, near-isogenic blends were compared with leafed and semi-leafless monocultures. Leaf types, varieties and their interaction were considered as fixed effects; replication, blocks, site-year and site-year by treatment interactions were considered as random effects.

To determine the effect of varietal mixture, all treatments, included near-isogenic pairs, non-isogenic pair, and leaf type monocultures, were analyzed by ANOVA, in which treatments were considered as fixed effects, and site-year and site-year by treatment interactions were considered random. Following ANOVA, contrasts were performed to compare the means of near-isogenic pairs (4 treatments) and non-isogenic pairs (12 treatments). Further contrasts were conducted to compare the means of one near-isogenic pair with the mean of non-isogenic pairs in each variety. [eg., CDC Amarillo (SL): CDC Amarillo (L) compared with non-isogenic pairs that contained CDC Amarillo as one of the varieties].

3.3 Results

In 2018, there was 103 mm of rainfall over the growing season. In comparison, the 2019 growing season received double that amount (Table 3.4). Temperatures were warmer in 2018 than they were in 2019 and the field experiments experienced drought conditions given the additional low levels of moisture. The Growing degree-days (GDD), (the sum of the daily mean temperature above 5°C) in 2018 showed the climate had greater heat accumulation than in 2019.

Table 3.4 Mean Temperature[*] and total precipitation[*] at the Kernen Research Farm during the 2018 and 2019 growing seasons.

	Mean Temperature (°C)		Total Precipitation(mm)		Growing degree-days	
	2018	2019	2018	2019	2018	2019
April			9.1	0.4		
May	14.1	9.7	35	4.4	283.5	169.0
Jun	17.3	16	19.9	84.8	362.3	330.3
Jul	18.7	17.8	31.1	67.7	423.5	398.5
Aug	17.1	15.4	17.1	20.3	374.6	322.7
Average	16.8	14.2				
Total			112.2	217.1	1443.9	1220.5

[*]Environment Canada

3.3.1. Variety characteristics of leafed near-isogenic lines and semi-leafless lines

ANOVA testing showed that the leafed NILs did not differ from the semi-leafless parents in disease severity or maturity (Table 3.5). Analysis of the disease severity data showed a random effect of the variety by site-year interaction (Table 3.5), indicating varieties responding to disease severity were inconsistent across sites. Due to growing conditions disease was more severe in 2018 than in 2019. Maturity results showed an effect due to variety.(Table 3.5) where CDC Dakota took 2-4 days longer to mature than other varieties. A significant random effect of the variety by site-year interaction being significant was also due to differences of temperature and rainfall amounts between the two years (Table 3.4).

Table 3.5. ANOVA table for disease severity and maturity as affected by monoculture (semi-leafless vs leafed), and variety in 2018 and 2019.

Source of Variation	DS	M
Variety (V)	0.410	**0.014***
Leaf type (LT)	0.113	0.792
V * LT	0.494	0.157
Block	-	0.345
Rep	0.156	-
Site-year (SY)	0.133	0.113
V * SY	**0.051***	**0.054***
LT * SY	0.309	NA

DS – disease severity; M – maturity

Pooled data of two sites in 2018 and three sites in 2019

3.3.3. Effect of leaf type blends on yield and agronomic performance

Effect of leaf type blends on yield and agronomic performance

Leaf type differed in disease severity, and the variety effect varied by site (Table 3.6). Disease levels in 2018 and 2019 differed significantly due to the different environments. Mycosphaerella blight and pea rust (*Uromyce viciae-fabae*) were the major diseases in 2018, with an average of 52% and 62% severity respectively. In the following year, Mycosphaerella blight and Aphanomyces root rot were prevalent, with an average of 31% and 43% severity respectively. Analysis of disease severity illustrated that the leafed pea type had a 6% higher infection than the near-isogenic leaf blend and the semi-leafless pea. Disease severity between the blends and the semi-leafless monoculture were similar.

Table 3.6. ANOVA table for light interception, lodging height index, crop biomass, and crop yield as affected by leaf type variety, and date after seeding in 2018 and 2019.

Source	Light Interception	Disease Severity	Lodging Height Index	Biomass	Yield
Variety (V)	0.905	0.565	**0.0027****	0.105	0.104
Leaf type (LT)	0.587	**0.045***	0.174	0.215	**<.0001*****
V * LT	0.917	0.639	**0.0116***	0.762	**0.0494***
Date (D)	0.053*	-	-	-	-
LT * D	0.562	-	-	-	-
V * D	0.361	-	-	-	-
LT * V * D	0.332	-	-	-	-
Rep	0.058	0.332	ns	ns	ns
Block	0.096	ns	ns	0.391	0.498
Site-year (SY)	0.242	0.136	0.368	0.115	ns
V * SY	0.266	**0.032***	0.061	0.357	0.301
LT * SY	0.323	0.376	0.153	ns	0.206

Pooled data of two sites in 2018, and three sites in 2019. ns, non-significant; *$p < 0.05$; ** $p < 0.01$; ***$p < 0.001$.

The interaction of leaf type by variety was significant for lodging height index (Table 3.6). Analysis of the interaction showed that the semi-leafless monoculture across varieties had a relatively high index or great lodging resistance(Figure 3.2). The leaf blends had a 9% lower lodging index compared with the semi-leafless monoculture of CDC Dakota. The lodging index of the blends of CDC Amarillo, CDC Centennial and CDC Striker did not differ from the semi-leafless monoculture but the leafed monoculture was 10% and 14% higher than both the leaf-type blends and the semi-leafless monoculture, respectively.
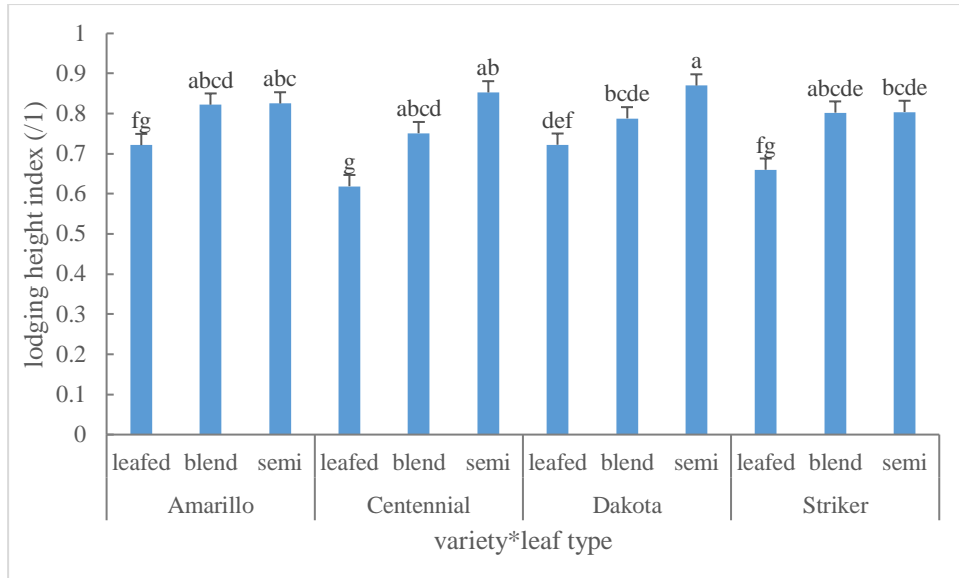
Figure 3.2. Interaction of field pea variety and leaf-type on lodging height index. Mean of two sites in 2018 and three sites in 2019. Index = 1 indicates no lodging. Columns with different letters representing the mean of lodging height index were significantly different in LSD$_{0.05}$. The error bar represents the positive standard error. Columns with common letters are not significantly different from each other.

Harvest yield differed by year. Average yield was 864 kg ha^{-1} in 2018 and 1802 kg ha^{-1} in 2019. There was a significant effect of variety by leaf-type interaction for crop yield (Table 3.6). In general, the 75:25 SL/L blend yielded similar to the semi-leafless and leafed monoculture treatments (Figure 3.3). The exception was CDC Centennial, in which the semi-leafless monoculture had a 13.4% greater seed yield when compared to the leafed monoculture, while the blends showed the yields to be statistically similar (Figure 3.3).
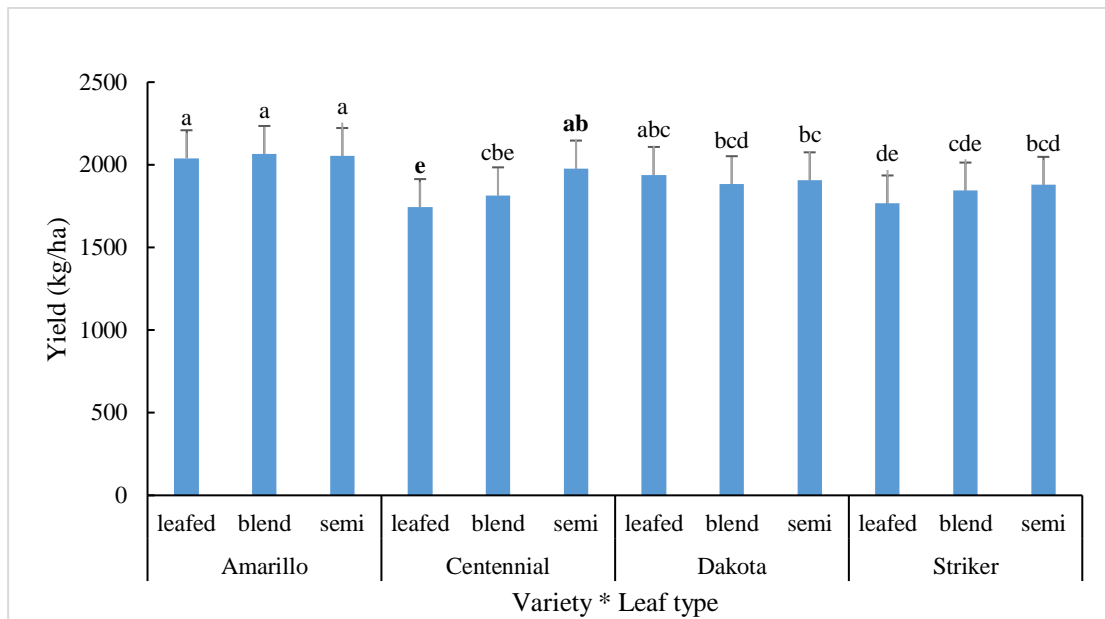
Figure 3.3 Interaction of variety and leaf-type on field pea yield (kg ha^{-1}). Mean of two sites in 2018 and three sites in 2019. Columns with different letters representing the mean of disease severity were significantly different in LSD$_{0.05}$. Columns with common letters are not significantly different from each other. The error bar represents the positive standard error.

3.3.4. Effect of leafed / semi-leafless variety mixtures on field performance compared to variety monocultures.

To determine the effect of varietal mixture, the mixture and variety monoculture were compared by contrasting the non-isogenic and near-isogenic blending pairs. The contrasts revealed no difference between the near-isogenic and the non-isogenic blending pairs for lodging height, disease severity, biomass, or seed yield (Table 3.7). The ANOVA showed significant difference of near-isogenic, non-isogenic, and monocultural treatments for lodging height index and yield. The difference between the mean disease severity of all treatments tended to be significant ($P < 0.10$). This also led to significant effects of site-year and treatment interactions for all variable responses (Table 3.7). The study analyzed the contrasts of the near-isogenic blends and non-isogenic blends for each site-year. The trends for individual site-years were similar to the combined site-years so the combined results are presented.

Table 3.7. ANOVA table for disease severity, lodging height index, crop biomass, and crop yield as affected by near-isogenic pairs and non-isogenic pairs when combined, and compared within variety[a]. in 2018 and 2019.

Source	Lodging Height Index	Disease	Biomass	Yield
Treatment (Trt)	<.0001***	0.099	0.8418	0.0005***
Contrasts				
Near-isogenic pairs (NIL) vs Non-isogenic-pairs (non-NIL)	0.987	0.891	0.909	0.815
CDC Amarillo NIL vs Non-NIL	0.905	0.769	0.458	0.250
CDC Dakota NIL vs non-NIL	0.680	0.831	0.903	0.791
CDC Striker NIL vs non-NIL	0.384	0.720	0.915	0.724
CDC Centennial NIL vs non-NIL	0.710	0.998	0.773	0.314
Random effects				
Site-year (SY)	0.107	0.173	ns	ns
SY*Trt	0.005***	<.0001***	<.0001***	0.009***

[a]pooled data, two sites in 2018, three sites in 2019
ns, non-significant; *p < 0.05; ** p < 0.01; ***p < 0.001.

The non-significant treatment differences for disease severity were likely due to all varieties in the study being susceptible or moderately susceptible to disease, with high disease years (Table 3.1). Blending leaf-type or variety did not significantly improve the disease resistance (P-Value = 0.099). However, for some blends, mixing a susceptible variety with one moderate-susceptible tend to slightly lower disease ratings when compared to the susceptible variety alone (Figure 3.4). For example, with CDC Centennial , the non-isogenic blends had a 3-7% reduction in disease severity compared to the near-isogenic blends (Figure 3.4).
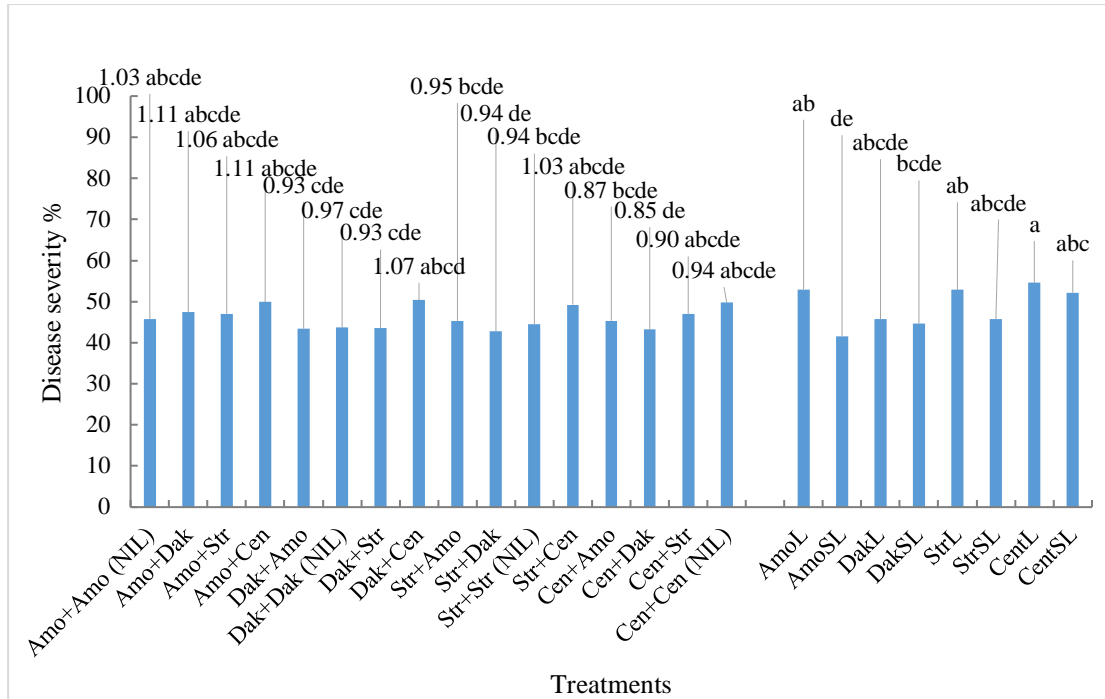
Figure 3.4 Disease severity for treatments of mixtures relative to monoculture (RDis) for near-isogenic blends (NIL), non-isogenic blends, semi-leafless monocultures (SL), and leafed monocultures (L) in 2018 and 2019.

Treatments listed as semi-leafless + leafed lines for CDC Amarillo (Amo), CDC Centennial (Cen), CDC Dakota (Dak), and CDC Striker (Str). Lowercase letters indicate the significant differences among treatments (LSD$_{0.05}$). Pooled data

There was no consistent yield advantage for non-isogenic blending pairs when compared to near-isogenic blending pairs across varieties (Table 3.8). The near-isogenic blends in CDC Amarillo and CDC Dakota had greater yield than some non-isogenic blends; however, the yield advantages were a result of high-yield genotypes, not the effect of variety monoculture.

The yield stability (*Wi*) data showed that the non-isogenic blends did not provide greater stability than the near-isogenic blends (Table 3.8). Neither single varieties nor varietal mixtures consistently increased relative yields in the mixture compared to monoculture (Table 3.8). The yield of near-isogenic and non-isogenic blends was generally intermediate to the monoculture (*RYld* closes to 1), regardless of variety (Table 3.8), even though high yielding CDC Amarillo and low yielding Centennial varieties were included in testing.

Table 3.8. Yield and relative yield of mixtures compared to monoculture (*RYld*) including ecovalence values (*Wi*) of near-isogenic blends (NIL), non-isogenic blends, semi-leafless monocultures (SL), and leafed monocultures (L) in 2018 and 2019.

SL+L in 75%/25% mixture	Yield[*] (kg/ha)	RYld (>1 is greater)	Ecovalence value Wi
Amo+Amo (NIL)	1628.9ab	1	10.52
Amo+Dak	1613.7abc	0.97	1.91
Amo+Str	1582.3bcd	0.97	3.91
Amo+Cen	1585.2cd	0.94	2.08
Dak+Amo	1542a	1.06	6.99
Dak+Dak (NIL)	1526.9abc	1	5.29
Dak+Str	1495.9d	0.98	3.76
Dak+Cen	1498.4abcd	1.03	0.87
Str+Amo	1517.7abcd	1.02	7.4
Str+Dak	1502.5abcd	1.05	13.36
Str+Str (NIL)	1471.1bcd	1.01	2.21
Str+Cen	1474cd	1.03	20.12
Cen+Amo	1576.7abcd	0.97	1.93
Cen+Dak	1561.6bcd	0.94	3.28
Cen+Str	1530.2cd	1	11.88
Cen+Cen (NIL)	1533d	0.95	4.48
Monocultures			
Amarillo L	1591.0	-	-
Amarillo SL	1641.5	-	-
Dakato L	1530.4	-	-
Dakota SL	1525.7	-	-
Striker L	1404.7	-	-
Striker SL	1493.2	-	-
Centennial L	1416.3	-	-
Centennial SL	1572.0	-	-

[a]Pooled data

Pea varieties and pair combination are shown as abbreviations: CDC Amarillo (Amo), CDC Centennial (Cen), CDC Dakota (Dak), and CDC Striker (Str). [*]Yields with different letters significantly different (LSD$_{0.05}$).

3.4 Discussion

Blending semi-leafless and leafed pea types improves lodging resistance, and reduces disease severity compared to the leafed monoculture. However, the leaf blends do not significantly increase yield. The agronomic and yield results suggest that blending leafed and semi-leafless peas have few advantages to growing semi-leafless pea types

only. The *RYld* results illustrated that the leafed pea in the near-isogenic blends had a similar yield productivity as the relative leafed line monoculture (*RYld*=1). This result is not unexpected as the near-isogenic leafed varieties genetically resemble the semi-leafless variety, differing only in leaf morphology. Kof et al. (2014) reported that although a leafed pea has greater chlorophyll content (1.5-fold per plant) and leaf area, semi-leafless intensive tendrils cover the photo-assimilating potential and result in a non-diminished final biomass and grain yield. The main effect of using leaf blends is to prevent yield loss from lodging.

The present study compared isogenic pairs of leafed and semi leafless pea genotypes with blends that were not isogenic and found that regardless of the isogenic status of the blend, the seed yield was similar to the semi-leafless parent. This study was conducted under a weed-free conventional cropping system. In contrast, Syrovy et al. (2015) under organic growing conditions reported that a leaf blend resulted in 156% and 18% more seed yield than the leafed and semi-leafless monocultures, respectively. The differences between studies could be due to the differences in cropping systems or simply a function of the single pair of unrelated crop varieties that Syrovy et al. chose, as the leafed variety used was a forage variety, not optimized for seed production. The author concluded the increase was due to improved canopy weed suppression and lodging resistance. The present study also found the blends improved leafed lodging resistance relative to the leafed parent, but this did not result in a yield increase.

Crop-weed competition may have caused differences in leafed pea yield in previous studies. Vasilakoglou and Dhima (2011) reported that leafed pea had reduced yield loss in weedy conditions, whereas, in weed-free treatments, the semi-leafless type had a 37% higher yield. Gollner et al. (2019) found leafed pea had lower weed pressure than leaf blends and semi-leafless pea types at the emergence stage, and a better photosynthetic efficiency resulted in the leafed pea producing the highest grain yield under organic cropping systems. In the current study, the leaf blend experiments were conducted under conventional management, and weed removal by herbicides may have negated any advantage of weed suppression by the leafed pea component.

Other studies have observed that leaf blends produced higher yield than monocultures in weed-free conditions. It has been attributed to the synergistic effect of genotypes, in which the high-yielding leafed component contributed to a higher yield. Zivanov et al. (2018) used a 50:50 SL/L ratio and showed that the leafed component had higher grain yield than the semi-leafless component at harvest, yet the yield of the mixture did not

differ from the leafed and semi-leafless monocultures. The present study used four near-isogenic leafed and semi-leafless pairs, which provided a more reliable determination for leaf blend yield and agronomic performance. Comparing the near-isogenic blends with the non-isogenic blends showed that leaf-type blends were not affected by genotype mixture in lodging height index, disease, or biomass. The *RYld* illustrated that the leaf blends, either near-isogenic or non-isogenic were generally intermediate to the monoculture. Nevertheless, the exhaustive comparison of near-isogenic and non-isogenic blends grown under multiple environments in the present study indicates that there is no yield advantage to leafed/semi-leafless blends under weed free conditions.

The effect of variety mixture in this study supported Finckh et al. (2000), who emphasized that variety mixtures can provide functional diversity for disease resistance. The *RDis* results showed a potential disease reduction indicating that mixing another more disease-tolerant variety may lead to slightly lower disease levels. Villegas-Fernández et al. (2021) showed a significant disease reduction from blended powdery-mildew resistant and susceptible varieties, in different ratios, and found that infection decreased with increasing proportions of the resistant types. Since most pea varieties are similar in disease resistance within Western Canada (Saskatchewan Variety Guide. 2021), disease control was not a priority in this study. Mixing varieties with better disease resistant ones can have a barrier effect that can be a low-input suitable disease control method to reduce yield loss.

To our knowledge, this study is the first to investigate mixing plant varieties and leaf morphological traits together. Our study found that the only advantage of mixing leaf types was to bring positive characteristics to the deficient variety in the mixture. This was observed by reduced lodging and lower disease severity in some mixtures when compared to the sole cropped variety. Likewise, Horner et al (2019) mixed two varieties supporting significantly different fungal and bacterial communities. The microbial activity of both varieties was stronger when leaf types were mixed than when grown as a monoculture, and both varieties produced slightly higher yield. Jackson and Wennig (1997) found that varietal mixtures in wheat, having complementary lodging-resistant and disease-resistant traits, significantly reduced lodging and disease severity compared to the susceptible corresponding variety. However, yield of the mixture tended to be intermediate compared to the pure stand when there was no significant positive interaction of competitive ability between components. Darras et al. (2014) grew semi-leafless pea in a two-way genotype mixture among three related and one non-related

variety. They showed no significant yield increase in the genetic mixture compared to the pure stands, regardless of weed or weed-free conditions. Neither yield nor crop competitive ability was enhanced by the genetic relatedness of the mixture variety.

3.5 Conclusion

This study investigated the effect of mixing varieties and leaf types in field pea, with four NILs. Blending semi-leafless and leafed pea in a 75/25% ratio resulted in reduced lodging of some varieties and small decreases in disease severity when compared to the leafed monoculture, but performance was equal to the semi-leafless monoculture. Including the leafed component did not result in the expected advantage of greater solar interception and synergistic yield increases when lodging was decreased. The results determined that the main benefit of using leaf blends is to prevent leafed pea yield loss due to lodging, but does not increase the overall yield in a conventional growing system. We compared near-isogenic and non-isogenic pairs and found no difference in lodging, disease severity, light interception, or yield indicating that the relation of the leafed and leafless varieties in the blend had no effect. The yield of the leaf blends was generally intermediate to the corresponding monocultures, either near-isogenic or non-isogenic.

4. Chapter 2: An optimal leaf blend for field pea yield and agronomic improvement

Abstract

Field pea has semi-leafless (SL) and leafed (L) types., Intercropping of these two types may improve yield by optimizing pea solar radiation interception, reducing lodging, decreasing disease and enhancing weed competition. However, an optimum blending ratio has not yet been established since previous intercrops mixed two leaf types from two separate genotypes. This study used four near-isogenic pairs (NIL) of pea genotypes differing only in leaf type to determine the optimal blending ratio of semi-leafless and leafed pea for the highest yield and competitive agronomic traits. The experiment was conducted at five locations. Four semi-leafless varieties with their related leafed NILs were intercropped in three mixing ratios: 50:50, 67:33, and 83:17 SL/L. For comparison, the leafed or semi-leafless types were also grown as monocultures. The results showed that the ratio of over 67% semi-leafless pea decreased lodging by 10% compared to the leafed monoculture. For disease, the 83:17 blend decreased disease infection by 4% compared to the leafed monoculture. However, the intercrops had no significant beneficial effect on light interception and biomass production. Regression analysis estimated that the 86/14 ratio produced the highest yield, which provided an 11% increase to the leafed monoculture but there was no increase compared to the semi-leafless monoculture. The intercrops composed of 14% leafed pea and 86% semi-leafless pea enhanced leafed lodging resistance comparable to semi-leafless, and prevented yield loss due to lodging; however, the improvement to the semi-leafless pea monoculture was minor. Further benefits may be realized if the intercrop combined a high yield potential leafed variety with a lodging resistant semi-leafless variety in a 14:86% leafed to semi-leafless ratio.

4.1 Introduction

Field pea is a major pulse and important cash crop in Canada, with exports totaling 3.2-3.94 million tonnes from 2017 to 2019 (Agriculture and Agri-Food Canada, 2019). Saskatchewan is the largest pea-growing province and contributed 2.3 million tonnes of production in 2017 (Government of Saskatchewan, 2018). Field pea is a crop that can take advantage of the growing plant protein market due to its relatively high protein

content of 21.3~24.7% (Ren et al., 2021). The value of the Saskatchewan pea crop in 2018 was estimated to be $464 million. In addition to economic benefits, pea provides nitrogen fixation, and breaks disease cycles through crop rotation.

Previous studies of pea grown with strong stemmed crops such as barley, wheat, or oat, resulted in reduced pea lodging and higher yield than when either crop was grown on its own (Podgórska-Lesiak & Sobkowicz, 2013; Pelzer et al., 2012; Shaw et al., 2020). In cereal-pea intercrops, the cereals support pea and reduce lodging while the pea adds nitrogen to boost the yield of the cereal crop (Pelzer et al., 2012). The cereal in the intercrop may suppress pea growth, with intercropped pea having a reduced number of pods, less seed, and smaller seed size (Živanov et al., 2018).

Semi-leafless and leafed pea are the same species but differ in leaf structure. In leafed pea, the leaf consists of a stipule, leaflets, and tendrils, whereas the semi-leafless pea leaf consists of only a stipule and tendrils. This modified leaf structure causes them to differ in field characteristics. Leafed pea was more competitive and suppressed weeds better than semi-leafless pea in weedy conditions (Harker et al., 2008). The leafed leaflet led to a greater leaf area than the semi-leafless tendrils, which provided suppression of inter-row weeds and greater canopy radiation interception (Wall & Townley-Smith, 1996). Armstrong and Pate (1994) found the leafed type had greater green area index, extended green area duration and maintained a high growth rate compared to the semi-leafless type. Several studies have shown that leafed pea overcame the semi-leafless pea in harvested yield (Gollner et al., 2019; Olle, 2017). In comparison, semi-leafless pea has greater lodging and disease resistance than leafed pea (Wang et al., 2006; Banniza et al., 2011). An intercrop of semi-leafless and leafed pea may optimize pea radiation interception, reduce lodging and disease, as well as improve weed competition in comparison with sole grown leaf types. Schouls and Langelaan (1994) first intercropped leafed pea with semi-leafless pea and reported a lodging resistance improvement and higher yield from the intercrop compared to pure leafed stands. Syrovy et al. (2014) compared leaf blend ratios of 25:75, 50:50, and 75:25 (semi-leafless/leafed) in organic cropping for weed control. The study reported that 50:50 blends reduced weed biomass by 19% compared to a semi-leafless monoculture. Moreover, the 75:25 leaf blend produced 18% and 156% higher yield than the pure semi-leafless and pure leafed stand, respectively. However, Gollner et al. (2019) investigated leaf blends in a 50:50 ratio under an organic cropping system and reported

that the highest yields were in the pure leafed stands, followed by the intercrops, and then the pure semi-leafless stands.

Mixing semi-leafless and leafed types in different ratios produced varied results for lodging, disease resistance and yield. For lodging and disease tolerance, Syrovy et al. (2014) found that increasing semi-leafless pea percentages compared to leafed pea linearly decreased lodging. In addition, the authors observed an increased percentage of semi-leafless pea increased disease severity in the mixture. In contrast, Wang et al. (2006) reported disease was negatively correlated with lodging resistance. To illustrate how blending ratio affects yield, Antanasovic et al. (2011) and Schouls and Langelaane (1994) seeded the intercrops in five ratios: 0:100, 25:75, 50:50, 75:25, and 100:0 (semi-leafless/leafed) and compared the ratios for yield and Land Equivalent Ratio (LER). LER is an index that describes the relative land area required under monoculture to obtain the same yield as under an intercrop (Mead & Willey., 1980). Antanasovic et al. (2011) found that the 75:25 ratio resulted in the highest LER, which provided a 9% and 39% yield increase compared to a pure semi-leafless and leafed stand. Schouls and Langelaan (1994) reported that the optimal semi-leafless percentage was 53-67%. Based on the previous information, the optimal ratio may range from a 50% or higher semi-leafless ratio where the semi-leafless crop is the supporting crop, the leafed crop is the supported crop. However, because all previous individual studies mixed two different genotypes with varying yield potential, lodging resistance, and vine length, a consistent blending ratio has not yet been determined.

To determine the optimal ratio for consistent leaf-blend performance, mixing ratios with a near-isogenic-line (NIL) would remove the confounding effect of genotype. NILs have been used to determine an alternative gene effect in phenology (Lanning et al., 2012), yield components (Arisnabarreta & Miralles, 2008), biotic stress (Jones et al., 2011) and abiotic stress (Venuprasad et al., 2011). In this case, an AFILA allele (*AF*), which controls leaflet development, was introgressed into a semi-leafless variety. The progenies were selected for leafed phenotype and repeatedly crossed with the semi-leafless parent. After five generations, the leafed lines genetically resembled the semi-leafless line with an *AF* allele and were called leafed NILs. The objective of this study was to determine an optimal ratio of near-isogenic semi-leafless and leafed intercrop to optimize pea disease resistance, lodging resistance, and yield.

4.2. Material and Methods

Site description

This study was conducted at the University of Saskatchewan Kernen Research Farm (latitude 52º 09', longitude 106º 32') in 2017 (1 site-year), 2018 (2 site-years), and 2019 (2 site-years) and the Rural Municipality of Blucher in 2019 (1 site-year). Both sites are located on a Sutherland series clay loam soil (Bradwell Dark Brown Chernozem; 10% sand, 40% silt, 50% clay).

4.2.1. Experimental design and management

The experiment was a factorial design with five blending ratios (0:100, 50:50, 67:33, 83:17, and 100:0 semi-leafless to leafed pea) with four semi-leafless pea varieties and their near isogenic leafed lines (CDC Amarillo, CDC Centennial, CDC Dakota, and CDC Striker). The proportions of the semi-leafless pea were higher than the leafed pea because mixtures of over 50% semi-leafless types were targeted to transfer the semi-leafless lodging resistance to the leafed pea (Syrovy et al. 2015). The field layout was an alpha lattice design with four replicates. Each semi-leafless parental variety was mixed with the related leafed NIL and planted in five ratio treatments, for a total of twenty treatments. The semi-leafless varieties were all bred by the Crop Development Centre (CDC) in Saskatoon. Four pea varieties were selected from three market classes to assess the constancy of the optimal ratio. CDC Amarillo and CDC Dakota are generally higher yielding than CDC Centennial and CDC Striker; further, CDC Centennial has greater lodging and Mycosphaerella blight (*Mycosphaerella pinodes*) disease susceptibility than the others tested (Saskatchewan Variety Guide, 2021).

The leafed NILs were bred by crossing semi-leafless parental lines with a leafed variety (CDC Sonata) to introgress the *AF* allele into their progenies using a backcrossing method (Devini DeSilva and Thomas Warkentin). In the F1 generation, the leafed progenies were phenotypically selected and backcrossed with the related semi-leafless parent to reduce the proportion of the leafed genome. After five generations, the leafed near-isogenic line expressed in relatively homozygous backgrounds that genetically resembled their related semi-leafless variety with the *AF* allele.

Pea was seeded in early May at a seeding rate of 88 plants m^{-2} in 3 x 6 m plots consisting of 6 rows, 30 cm apart. Seed of the intercrops were mixed prior to planting and seeded using a cone plot seeder. TagTeam® granular inoculant (*Penicillium bilaii* and *Rhizobium leguminosarum*) (Novozymes North America Inc., Franklinton, North Carolina, U.S.A.) was applied with the seed at a rate of 4.6 kg ha^{-1}. Monoammonium phosphate ($NH_4H_2PO_4$) was also applied with the seed to supply 16.5 kg ha^{-1} P_2O_5 and 3.85 kg ha^{-1} N. The site was managed similar to a commercial farm. Plots were treated with Odyssey® herbicide (*imazamox 35% a.e. + imazethapyr 35% a.e.*) (BASF Canada Inc., Mississauga, Ontario, Canada) for weed control at the 3-leaf stage using a rate of 30 g ai ha^{-1}. The plots were desiccated with Reglone® Ion (*Diquat ion 20% a.e.*)(Syngenta Canada Inc. Guelph, Ontario, Canada) at the R7 growth stage.

4.2.2. Data collection

Population density was measured three weeks after crop emergence by counting plants in 1 m^{-2} quadrats, 50 cm from the plot edge in both the front and back of each plot. At the beginning of canopy closure, canopy light interception was measured using two sensors. Under the canopy, a LI-191R Line Quantum Sensor (*LI-COR Inc., Lincoln, Nebraska, U.S.A.*) was inserted into the center two rows perpendicular to the row direction and measured photosynthetically active radiation (PAR; µmol s^{-1} m^{-2}) integrated over a 1-meter length. Above the canopy, a LI-200R Pyranometer (*LI-COR Inc., Lincoln, Nebraska, U.S.A.*) simultaneously measured the PAR above the canopy. The canopy intercepted PAR rate is the PAR below canopy divided by the real-time PAR above canopy. The rate of light interception was calculated by:

Intercepted PAR = 1 – (PAR below canopy / PAR above canopy)

Disease severity was visually rated at the pod filling stage (R4 stage). Five random plants were collected from the entire plot. The percentage of infection was inspected by comparing each plant's symptoms on stems and leaves with an incremental scale, which has been described previously (Chiang et al., 2014). Lodging was measured at the beginning of maturity (R5 stage). Canopy height and plant length were measured on five random plants in each plot with a meter stick. The lodging height index was

calculated by dividing the canopy height by the plant length with a lower index indicating higher lodging severity (Stelling, 1989).

Crop biomass was collected when pea was approaching maturity (R7 stage). Above-ground plant material was sampled in 0.25 m^{-2} quadrats, 50cm from the edge in both the front and back of each plot. The collected samples were dried in an oven at 70˚C for 48 hours to obtain dry biomass weight. Seed yield was obtained with a plot combine following desiccation when pod moisture was below 30%. The harvested seed was dried by forced air for 48 hours to obtain an equilibrium moisture. The seed was cleaned and weighed.

4.2.3 Statistical Analysis

The Coefficient of variation (CV) was calculated in the pre-analysis to ensure the consistency of the measurements for all response variables. Response variables with a CV value <30% is acceptable for agricultural research (Kwanchai, 1984). Data were tested for homogeneity of variances prior to analysis using the Levene's test in the General Linear Model procedure. The variance of site-year was heterogeneous for all variable responses, except for disease severity. Data were analyzed with analysis of variance (ANOVA) using the MIXED model in SAS 9.4 version (*SAS Institute Inc., Cary, NC, USA*).

To compare the leaf blending ratios, ratio, variety, and their interaction were analyzed as fixed effectswhereas replication nested in site-year, block nested in replication, site-year, and the interaction of site-year with fixed factors were assigned as random effects. Yield was also analyzed by quadratic regression, where the percentage of semi-leafless peas was assigned as an independent numeric variable. REPEATED statements in the MIXED analysis were used for spatial variability and to adjust for heterogeneity of variance (Stoloff, 1970). To measure the spatial variability, the distribution of each plot was converted to a matrix by inputting east and north locations of each plots in the site. The exponential covariance structure was used to model the matrix for spatial variation (Marx & Stroup, 1993). The *group=option* adjusted the site-year covariance. Treatment means were separated using Fisher's protected least significant difference (LSD) test. Treatment effects were declared significant at *P*<0.05; however, some trends are reported at *P*<0.1. A significant interaction of site-year and variety occurred for pea

yield but separating site-year results showed only differences for CDC Centennial., As the trends and rankings of the ratios were the same, yield was analyzed by combining site-years.

4.3. Results and Discussion

4.3.1. Climate and growing conditions

The climate varied throughout the three growing seasons (Table 4.1). The precipitation in 2018 was lower than in 2017 and 2019, while the temperatures were highest in 2017.

Table 4.1: Mean Temperature and total precipitation at the Kernen Research Farm during the 2017 and 2019 growing seasons.

	Mean Temperature (°C)			Total Precipitation(mm)		
	2017	2018	2019	2017	2018	2019
April	4.3	-1.0	4.8	18.4	9.1	0.4
May	12.1	14.1	9.7	46.3	35	4.4
Jun	16.1	17.3	16	30.9	19.9	84.8
Jul	19.6	18.7	17.8	25.5	31.1	67.7
Aug	17.8	17.1	15.4	25.2	17.1	20.3
Average	14.0	13.2	12.7			
Total				146.3	112.2	177.6

Sources: Environment Canada.

4.3.2. Light Interception

There was a significant date-by-variety interaction (Table 4.2) as varieties differed considerably in their rate of canopy closure. The light interception was modeled with a quadratic polynomial regression model (Figure 4.1). The CDC Striker canopy initially had a greater light interception than all other varieties, and this trend continued to be greater than CDC Dakota and CDC Centennial. The CDC Amarillo canopy development was similar to CDC Dakota in the early stages but approached CDC Striker later in the season. CDC Dakota had slightly higher PAR than CDC Centennial early in the growing season, however, that trend reversed as the season progressed.

The current study found that the leaf mixing ratio did not affect photosynthetic active radiation (Table 4.2) meaning that the rate of canopy closure and the photo-assimilation area of the leaf ratios were similar. Kof et al. (2004) reported that though semi-leafless

pea has reduced leaflets, the stipules and tendrils became the extended photosynthetic area and compensated by accumulating higher chlorophyll content, therefore the plant productivity of semi-leafless pea did not decrease with the leaf area.

Table 4.2: ANOVA table light interception, lodging height index, disease severity, crop biomass, and seed yield as affected by variety, blending ratio, and days after seeding (date) in 2017 and 2019.

Source	Light Interception	Lodging Height Index	Disease Severity	Crop Biomass	Crop Yield
Variety (V)	0.14	0.18	0.37	0.13	0.0844
Ratio (R)	0.78	**0.03***	**0.09**	0.85	**<.0033****
V * R	0.22	0.09	0.38	**0.04***	**0.0049****
Date (D)	<.0001*	-	-	-	-
V * D	**0.06**	-	-	-	-
R * D	1	-	-	-	-
V * R * D	0.4	-	-	-	-
Block	0.02	-	0.11	-	-
Rep	0.03	0.23	0.39	-	0.22
Site-year (SY)	0.12	-	0.50	-	-
V * SY	0.09	0.21	0.08	-	0.01
R * SY	0.09	0.12	-	0.13	0.05

Pooled data. One site in 2017, one site in 2018 and three sites in 2019

*, ** Source of variation significant at 0.05 and 0.01 P level, respectively;
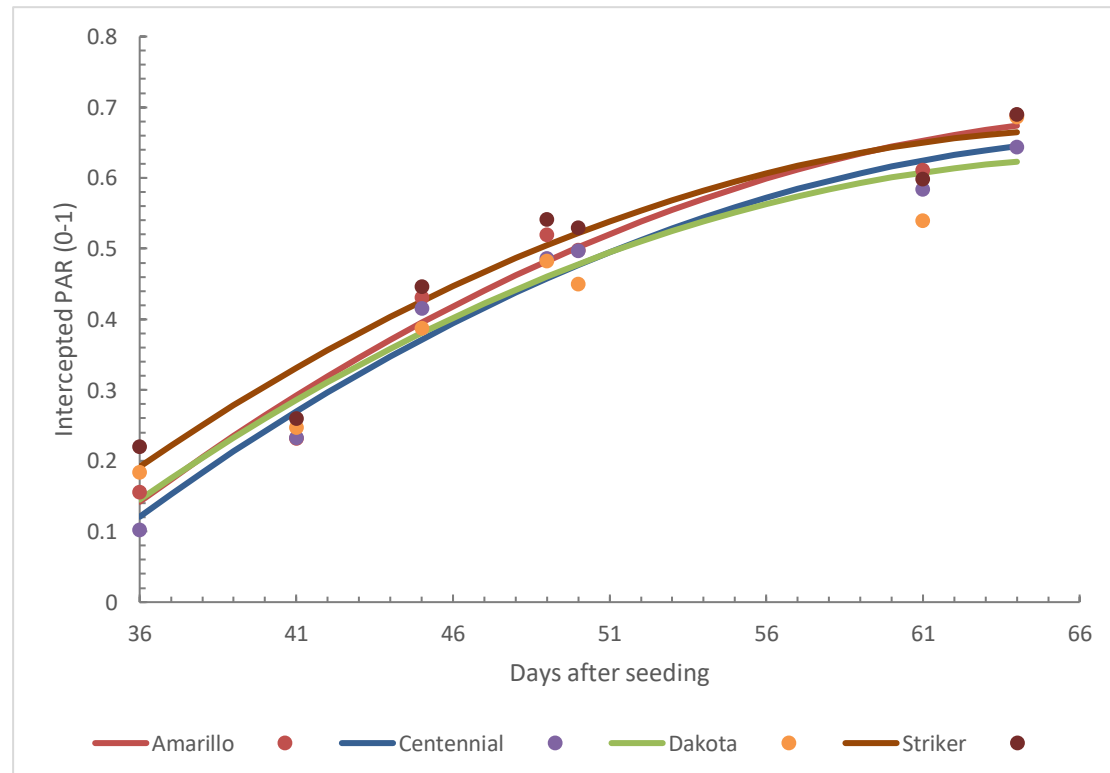
Figure 4.1: Canopy light interception of four field pea varieties during early vegetation and pod stages. Mean of one site in 2017, two sites in 2018, and two sites in 2019.

4.3.3. Lodging Height Index

There was a significant ratio effect on the lodging height index in this study (Table 4.2). The 66:37 ratio decreased lodging compared to the leafed monoculture by 10%. A trend of lodging height index showed that lodging in the intercrops tended to be severe when the leafed proportion exceeded 33%; lodging tended to increase as the leafed ratio increased (Figure 4.2). The lodging severity of the low leafed ratio is similar to the findings of Schouls and Langelaan (1994), who reported that the 25%~33% L and 75%~67% SL mixtures substantially reduced lodging compared to the sole leafed monoculture.
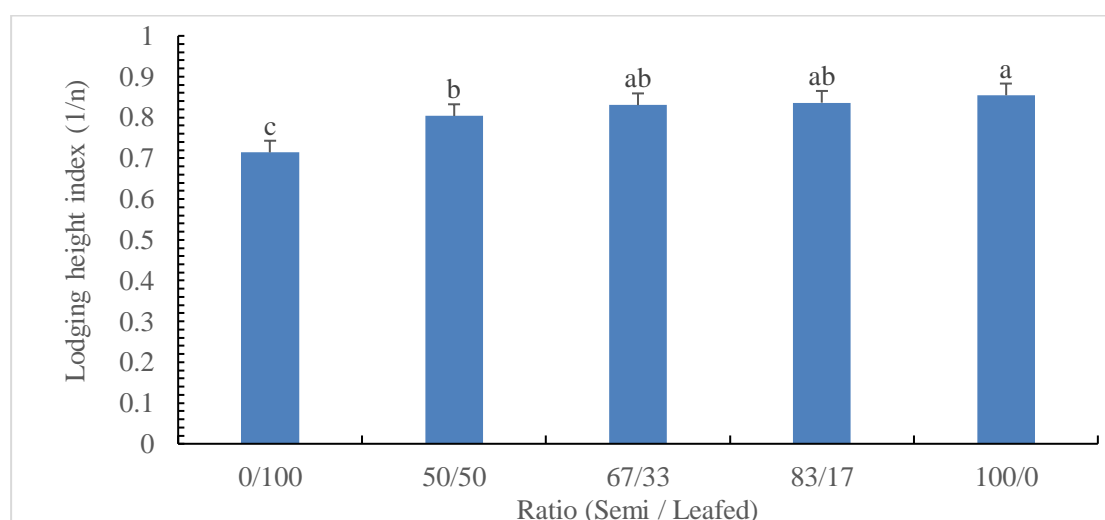
Figure 4.2. Effect of leaf-type blending ratio on lodging height index. Mean of one site in 2017, two sites in 2018, and two sites in 2019. Columns with different letters representing the mean of disease severity were significantly different. The error bar represents the positive standard error in LSD$_{0.05}$.

4.3.4. Disease Severity

The difference between the mean disease severity of the mixing ratio trended to be significant (Table 4.2). *Mycosphaerella* blight and pea rust (*Uromyce viciae-fabae*) were the major diseases in 2018 whereas *Mycosphaerella* blight and root rot (*Fusarium spp.*) were observed at the sites in 2019. Three leaf-type blends had relatively low

disease severities which developed a 4% lower infection than the leafed monoculture (Figure 4.3). Several authors have observed that *Mycosphaerella* blight was positively correlated to lodging timing and severity (Banniza et al., 2011; LeMay et al., 2016). The pathogens in lodged, compacted canopies are exposed to higher humidity, which is conducive to disease development (Wang et al., 2006). Hence, sole leafed pea first lodged, and infections proceeded upward to the mid and top leaves resulting in greater severity in the leafed monoculture compared to the blends and semi-leafless monoculture.
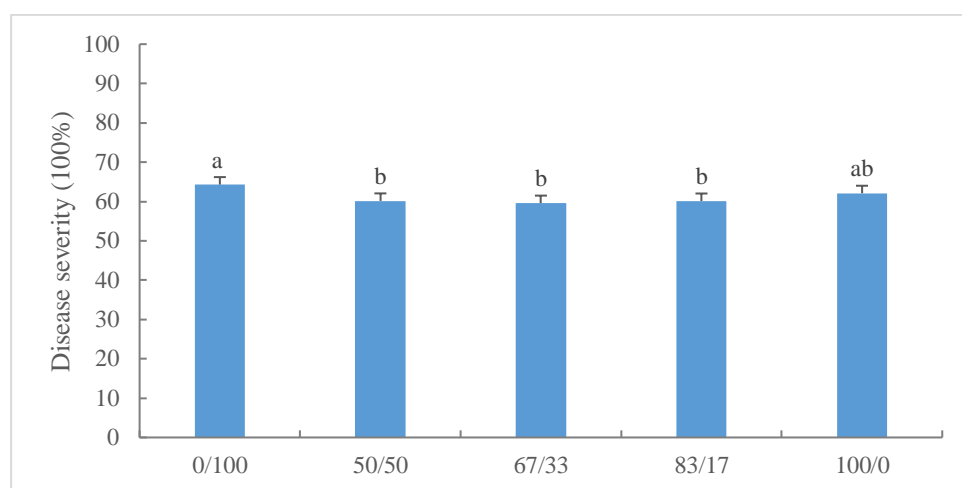
Figure 4.3. Effect of leaf-type blending ratio on disease severity. The ratios were listed as semi-leafless + leafed peas. Mean of one site in 2017, two sites in 2018, and two sites in 2019. Columns with different letters representing the mean of disease severity were significantly different in $LSD_{0.05}$. The error bar represents the positive standard error in $LSD_{0.05}$.

4.3.5 Biomass

The interaction between the ratio and variety tested significantly affected biomass weight (Table 4.2). In CDC Centennial, the leafed monoculture produced 11% lower biomass than the semi-leafless monoculture and the 67:33% (semi-leafless/leafed) blend. There was no difference in biomass among the blends or between the leaf types within all other varieties. The anticipated higher biomass weight in the blends was not observed, since the two leaf types do not differ in plant productivity. Harvey (1977) compared leaf types with near-isogenic lines and found that the reduced leaf area of the semi-leafless type did not reduce dry weight. Goldman and Gritton (1992) compared

near-isogenic leafed and semi-leafless pea and observed that leafed pea developed a larger total leaf area but had a lower total leaf weight.
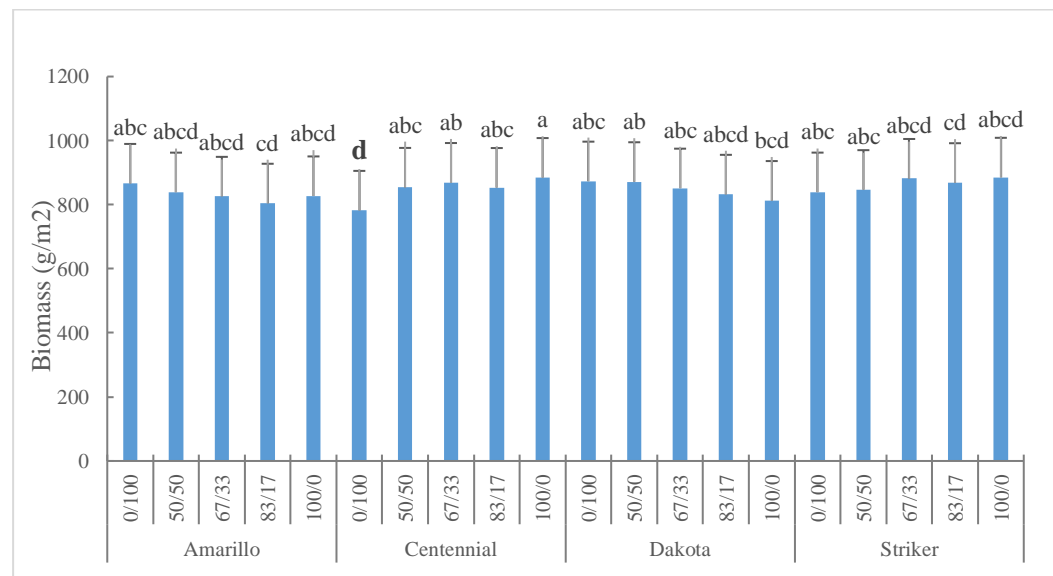
Figure 4.4. Interaction of variety and leaf-type blending ratio on field pea biomass. Mean of five site-years, one in 2017, two in 2018, and two in 2019. Columns with different letters representing the mean of disease severity were significantly different in LSD$_{0.05}$. The error bar represents the positive standard error in LSD$_{0.05}$.

Treatments were listed as semi-leafless + leafed pea for CDC Amarillo (Amarillo), CDC Centennial (Centennial), CDC Dakota (Dakota), and CDC Centennial (Centennial).

4.3.6 Yield

There was an interaction of variety and ratio for seed yield (Table 4.2). The leafed monoculture had significantly lower yield than the intercrops and the semi-leafless monoculture for CDC Amarillo, CDC Centennial, and CDC Striker. The 83:17 SL/L intercrop produced the highest yield of the blends, providing a 10% yield increase compared to the leafed monoculture but there was no yield difference compared to the semi-leafless monoculture. Maximum yield is a critical feature for optimal blending ratios; however, the ratios having the highest yield varied with variety. The 67/33 ratio in CDC Centennial optimized yield; meanwhile, the 50:50 ratio in CDC Dakota, the 83:17 ratio in CDC Amarillo, and the 100:0 ratio in CDC Striker resulted in the highest yield.

A quadratic regression ($P<0.001$), which combined all varieties, was developed to predict the variation in yield response of the blending ratios (Figure 4.5). Regression analysis revealed that the highest yield was the 86:14 ratio, providing an 11% yield increase in the leafed monoculture, yet no yield difference in the semi-leafless monoculture (Figure 4.6). Yield results corresponded with lodging showing a similar trend.
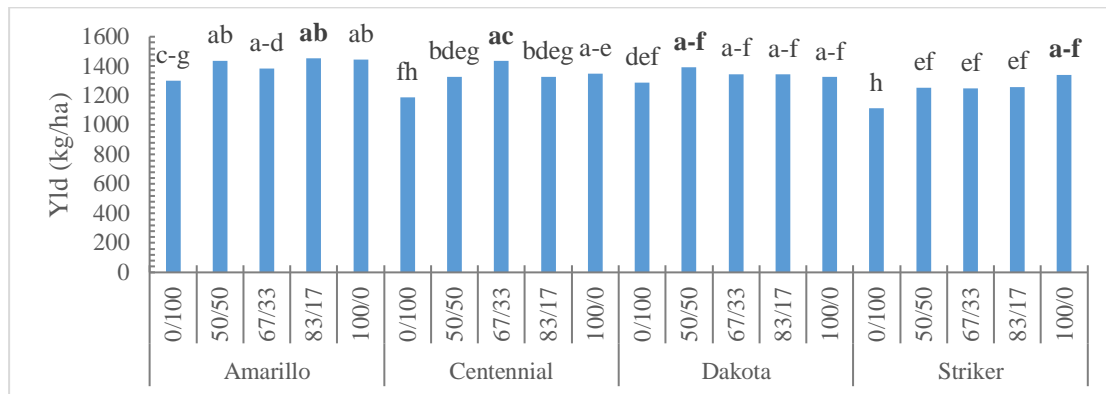
Figure 4.5. Interaction of variety and leaf-type blending ratios on yield (kg ha^{-1}). Mean of five site-years, one in 2017, two in 2018, and two in 2019. Columns with different letters representing the mean of disease severity were significantly different in LSD$_{0.05}$. The error bar represents the positive standard error in LSD$_{0.05}$.

Treatments were listed as semi-leafless + leafed pea for CDC Amarillo (Amarillo), CDC Centennial (Centennial), CDC Dakota (Dakota), and CDC Centennial (Centennial).

$$y = -0.0186x^2 + 3.216x + 1222.9$$
$$p=0.001$$
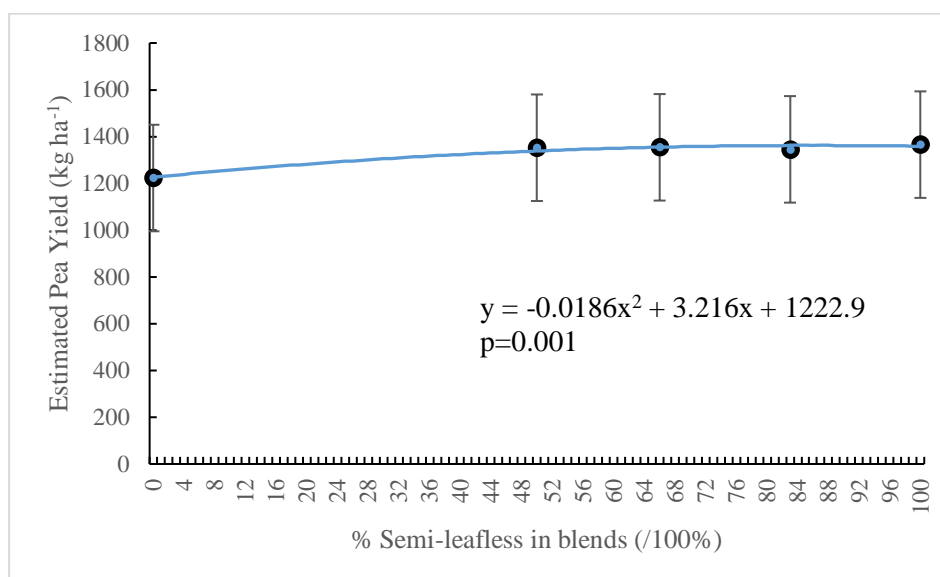
Figure 4.6. Regression of semi-leafless/leafed ratio on predicted yield based on the combined varieties. Mean of five site-years, one in 2017, two in 2018, and two in 2019.

The study used four pairs of near-isogenic lines and found that the optimal mixing ratio is in the range of 67~86% semi-leafless and 33~14% leafed pea. This would provide a relatively lodging-resistant canopy and that amount of leafed portion would not reduce yield compared to the semi-leafless monoculture. The anticipated yield benefits by blends with semi-leafless pea were not achieved in the present study. It could be due to the current study being conducted under weed-free conditions, which could lessen the leaf-blending advantage of crop competition. Syrovy et al. (2015) found that a 25% leafed CDC Sonata mixture with a 75% semi-leafless CDC Dakota improved leafed lodging resistance in an organic cropping system. The author reported that the leaf-type mixture led to weed and lodging reduction, with a 176% increase of leafed yield in the mixture compared to the leafed monoculture and a 22% increase compared to the semi-leafless monoculture. In contrast, Gollner et al. (2019) showed that the leafed monoculture had a higher yield than the leaf blends and the semi-leafless monoculture in organic systems. This may be due to the increased nitrogen fixation and photosynthetic efficiency of the leafed pea. In the current study, weeds were controlled by herbicides in early vegetative stages, thus early weed competition among these leaf types was excluded. Previous research in conventional grown field pea has also found that leafed pea can have a higher yield potential than semi-leafless pea when no lodging occurs (Mikic et al., 2011; Stelling, 1989; Schouls & Langelaan., 1994).

4.4. Conclusion
The study compared three ratios of the near-isogenic leafed and semi-leafless mixtures and compared them to their monocultures in four genotypes. It agrees the hypothesis that an optimal blending ratio provided good lodging resistance, disease tolerance, and high yield. Preventing and reducing lodging is the priority for optimal yield. Blending >67% semi-leafless and <33% leafed pea improved lodging resistance compared to the leafed monoculture. For disease, the 83/17 blend reduced disease infection by 4% compared to the leafed monocultures. The light interception showed that the leaf-type blend's photo-assimilation area during canopy closure was similar to the semi-leafless monoculture. The biomass determined that the crop productivity

among leaf types did not differ. The optimal ratio of 86% semi-leafless combined with 14% leafed pea provided an 11% yield increase compared to the leafed monoculture, which can relatively mitigate lodging. However, the study determined no improvement by leaf-type blend for yield compared to semi-leafless monoculture. It could be due to the current study being conducted in weed-free conditions, which decreases the leaf-blending advantage of crop competition. Future studies may benefit from the mixture of 14% leafed and 86% semi-leafless intercrops in an organic system.

5. Chapter 3: Precise quantification of crop lodging using UAV measures of canopy height reduction

Abstract

Unpiloted aerial vehicles (UAV) in research enables agricultural scientists to use high-resolution sensors to precisely measure plant phenotypes. UAV measured plant height has typically been quantified using a digital elevation map (DEM) produced by UAV orthomosaic. Lodging is a severe agricultural problem in field pea production, and a concerted effort is needed to improve lodging assessment. The overall objective of this study was to 1) quantify canopy height and plant length from UAV-based DEM and multi-spectral image, and 2) compare the lodging assessments with manually measured lodging index and image-derived height reduction. Pea images were collected by a UAV mounted multi-spectral camera and were processed to create DEM and multi-spectral images. A model in ArcMap software used the images to determine the plant elevation, the soil-surface elevation, and the normalized canopy height model (CHM). The image-derived CHM from 56 to 92 days after seeding (BBCH 60 - BBCH 89) was highly correlated with the measured height ($r^2 = .884$). The CHM variation shows a quadratic trend for pea lodging. The study analyzed the canopy height reduction from the image estimation and the lodging index from the ground measurements to the lodging assessment. The UAV derived lodging index was more precise than ground reference measurements. This resulted in significant treatment effects between pea variety mixtures with different lodging potential.

5.1 Introduction

Unpiloted aerial vehicles (UAV) enable agricultural scientists to use high-resolution cameras to precisely measure plant physiological features and replace tedious, time-consuming data collection and visual ratings. UAV imaging is a non-destructive novel phenotyping approach that provides high-throughout, precise, and standardized data (Yang et al. 2017).

Plant height is a typical phenotyping trait which can be determined and quantified from crop elevation maps. A previous study successfully used LIDAR, ultrasonic, and three-dimensional (3D) sensors in UAV-imaging to measure crop elevation (Wang et al., 2018). The plant heights were calculated as the difference between sensor observed elevation and the ground elevation. The results showed that the canopy heights derived

from the images had a strong correlation (r>0.9) with the ground measurements in sorghum (Wang et al. 2018). Digital Elevation Maps (DEM) imaged by UAV were initially used in landscape geographic references to measure the elevation of objects (i.e. terrain, plants, buildings) on the earth's surface (Peckham et al. 2007). Within the DEM, Digital Surface Models (DSM) and Digital Terrain Models (DTM) are composed. DSM is the elevation of a particular object on the DEM map, while the DTM is the elevation map referring to the bare earth reference (Joseph and Gyozo, 2007). Jiang et al. (2018) reported that maximum plant heights, extracted from multispectral maps and DEM, were strongly correlated to manual height measurements in cotton (r^2> 0.89). Sarker et al. (2020) derived peanut heights as the difference between DSM and DTM, which were highly correlated to manual measurements (r^2=0.953).

Lodging is a severe agricultural problem in field pea production. Lodging causes bending stalks (stem lodging), which further enhances disease infection, reduces plant photosynthesis, and increases harvesting difficulty (Singh et al., 2020; Schoul & Langelaan., 1994; Banniza et al., 2011; Zhang et al., 2006). Lodging has been found to cause 74% yield loss in some field pea varieties (Amelin & Parakhim, 2003). In light of this, concerted efforts are needed to enhance lodging research by improving lodging assessment.

In agricultural research, lodging is usually evaluated visually and graded with a 0-9 scale where 0=no lodging and 9=completely flat. However, ratings may be difficult and errors unavoidable, especially after assessing hundreds of plots or switching personnel doing the assessment (Sarker et al., 2020). Another method to measure lodging is to measure the plant height relative to the height of the crop canopy (Stelling, 1989). Plant height is the length of the stem from the aboveground portion to the tip of the plant shoots, whereas canopy height is the height from the soil surface to the top of the canopyr. A lodging index can be calculated by dividing the canopy height by the plant length. Although the lodging index is relatively accurate for lodging measurement, the method is destructive and can be time and labor consuming.

Several studies have assessed lodging severity in rice (Yang et al., 2017), maize (Chu et al., 2017), and spring wheat (Singh et al., 2019) using UAV imaging, where the lodging was measured as the difference of the pre- and post-lodging DEM. Singh et al. (2019) reported a high correlation (r=0.5~0.6) between visual rating and image estimated lodging in a ten-thousand plot wheat breeding study. In the current study, we hypothesized that field pea lodging severity derived from DEM by UAV-multispectral

imagery would be a more accurate lodging assessment than manual measurement. This study's overall objective was to 1) obtain canopy heights and plant length from UAV-based DEM and multispectral images, and 2) compare the lodging assessments from measured lodging index and image-derived lodging index.

5.2 Materials and Methods

5.2.1. Experiment Design and Management

The experiment used the same field trials as in Chapter 2 and the details have been described in 4.2.1.

5.2.2 Data Collection

Ground measurements

Data for canopy heights and plant lengths were measured with meter-sticks on the same day as the digital imaging was done. Five random plants were measured at 75-79 BBCH, 61- 82 days after seeding (DAS) in each plot. A lodging index calculated the angle of the main stem (Stelling, 1988):

Lodging Index = Canopy height / Plant length

Drone image acquisition

Ground Control Points (GCP) were installed prior to trial planting on bare ground in each corner of the experimental area to represent zero height. The geolocation of each GCP was determined using a Real-Time Kinetic (RTK) GPS. The DJI Matrix 600 platform was used to acquire aerial images, with the MicaSense Red-Edge camera in 2018 and the Hi-Phen camera in 2019. The images were collected at a height of 20 m with a 75% overlap for the experimental plots. Since pea lodging usually occurs after the BBCH51, the weekly UAV flights started 56 DAS to 91 DAS (Table 5.1).

Table 5.1. Dates and days after seeding for Unpiloted Aerial Vehicle (UAV) flights and manual height measurement for field pea plots in 2018 and 2019.

	UAV Flights Dates (DAS)	Manual Height Measurement Dates (DAS)
2019-site1	56,62,69,72,77,80	62
2019-site2	61,71,86	61

| 2019-site3 | 82,84 | 82 |
| 2018-site1 | 58,66,70,77,83,91 | 70 |

Imaging processing and data extraction

The multispectral images were pre-processed with Pix4D software (*Pix4D S.A. Switzerland*), which matched the GCP and calibrated the overlaps to create a stitched orthomosaic map. The 2D view DEM was created using structure from motion photogrammetry software processing the captured point clouds. The spatial resolution for DEM and orthomosaic was 2.96 cm/pixel. Then, they were loaded onto the ArcMap software Version 10.1.6 (*Esri. Redlands, California, U.S.A.*), which was used to apply the vegetative index and extract heights from the images.

Plot and Soil Background Segmentation

Image segmentation was used to classify the pea plots and the adjacent soil surface. To accomplish this, polygons were manually drawn on the map to annotate the plots and adjacent soil surface. Each plot polygon and relative soil polygon was identified by the plot number, and all polygons were grouped into a shapefile (Figure 5.1).
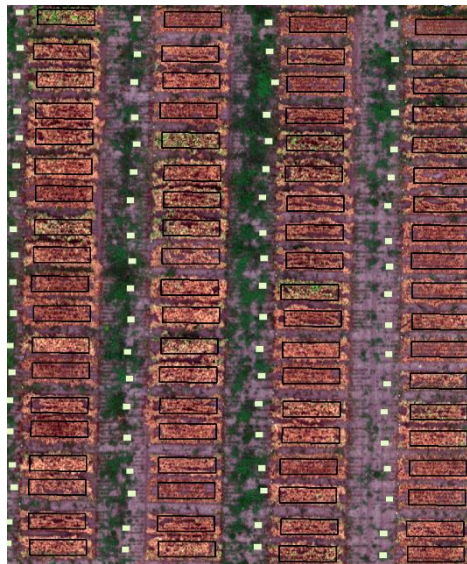
Figure 5.1: Multispectral map of field pea trial at the maturing stage, with the annotated plant (black) and soil (yellow) polygons. An example of an orthomosaic image in which the black rectangles represent the portion of the plots utilized for image analysis and

the adjacent white rectangles represent the soil polygons used to determine ground elevation for each individual plot.

Threshold layer

Normalized Difference Vegetation Index (NDVI) and Modified-Green Soil-Adjusted Vegetation Index (MGSAVI) were used to precisely exclude the soil from the plant annotation for different pea stages (Figure 5.2). When the canopy was at maximum density at BBCH 60, NDVI measured the plant material, and pixels less than 0.2 index were excluded. When plants were drying, MGSAVI replaced NDVI to achieve separation. MGSAVI is a change of the red band to the green band based on Soil-Adjusted Vegetation Index (SAVI). Gitelson et al. (1996) reported the maximum of Chlorophyll-A absorption was around 520 nm to 630 nm within the green band during the maturing and senescing stages. Within the index, the L parameter in the MGSAVI was at 0.5, and indexes less than 1.7 were excluded. The L parameter is the soil conditioning index, which improves the sensitivity of NDVI to the soil background.

$NDVI = (NIR - Red) / (NIR + Red)$

$MGSAVI = (NIR - Green) / (NIR + Green + L) + (1 + L)$
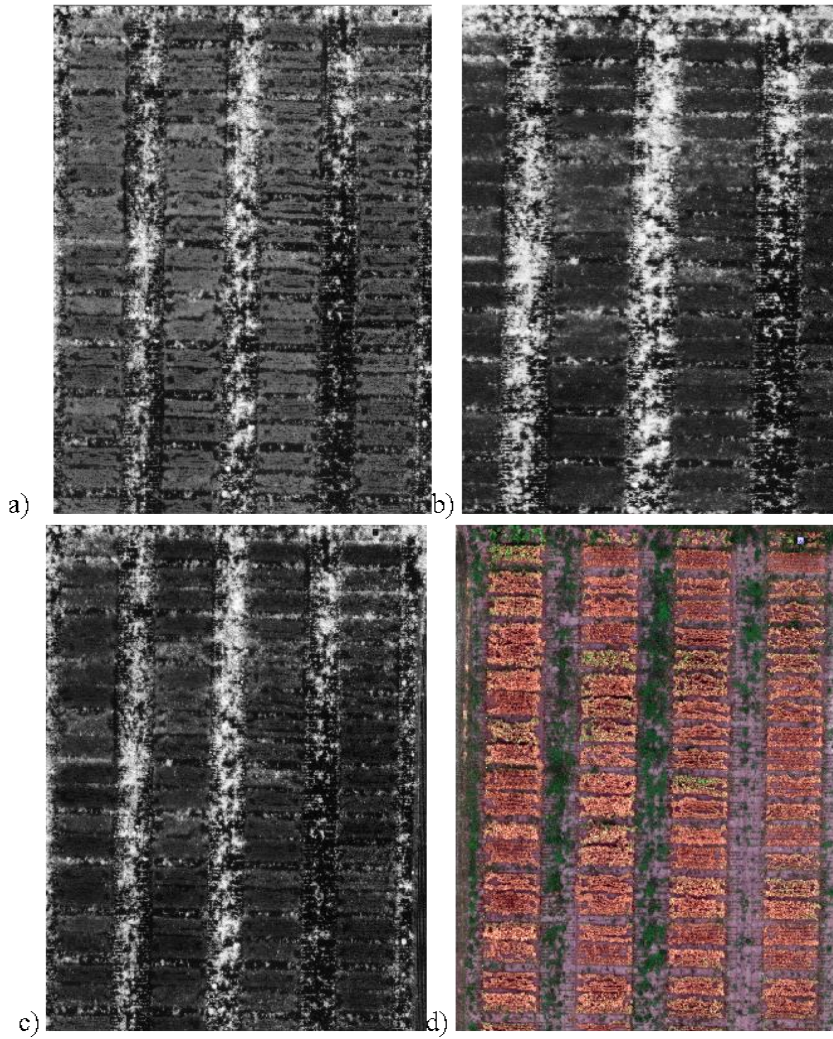
Figure 5.2: a) MGSAVI b) NDVI c) SAVI d) RGB raster layers in the field pea plots at BBCH 85 stage.

Canopy Height Extraction

A workflow shows the steps of the normalized canopy height model (CHM) extraction from DEM, vegetative raster layers, and segmented polygons (Figure 3). Flow chart A shows the functions used to process the images in ArcMap, and the B list shows the output layers derived from the functions. In chart A, there were three types of flow paths: the input sources (blue circle), the functions (orange rectangle), and the output layers (green circle). MGSAVI, DEM, Plant Polygons, and Soil Polygons were the input sources.

MGSAVI was imported into the 'Reclassify' function which separated and sorted the pixels in the MGSAVI to different ranges based on pixel value. The pixels above the

1.7 index MGSAVI layer were plant pixels, and these pixels were converted to a value of 1. In contrast, the pixels below the 1.7 index were biased pixels and their value was removed. As a result, MGSAVI was separated into a binary index, 1 or NoData, and converted to a plant feature mask called MGSAVI_removed_bias (Reclass MGSAVI). The DEM and the plant mask were subsequently imported into the Raster calculator function. The Raster calculator function combined the mask and the DEM, with the mask covering the DEM, and their pixel values were multiplied together. As a result, the elevation value of the DEM's plants were retained and the elevation values of bias were excluded. The newly produced layer was the plant DSM, also called MGSAVI_thresold_DEM.

Next, the plant polygons were applied for the plant DSM to segment plots in the map and imported into the 'Zonal Statistics as Table' function. Finally, the mean, mode, and area of pixel values within each plant polygon were calculated resulting in the Plant_Data Table**.** Alongside the plant elevation extraction, there was a path to adjust soil elevation. The soil polygons and the DEM were imported into the' Zonal Statistics as Table function', which calculated the mean, mode, and area of pixel values within each soil polygon and resulted in a table, called Soil_Data. These two tables provided elevations for the plants and soil surface for each experimental plot, and the mean value of the soil surface was subtracted from the mean plant elevation value to calculate the normalized canopy height model (CHM).
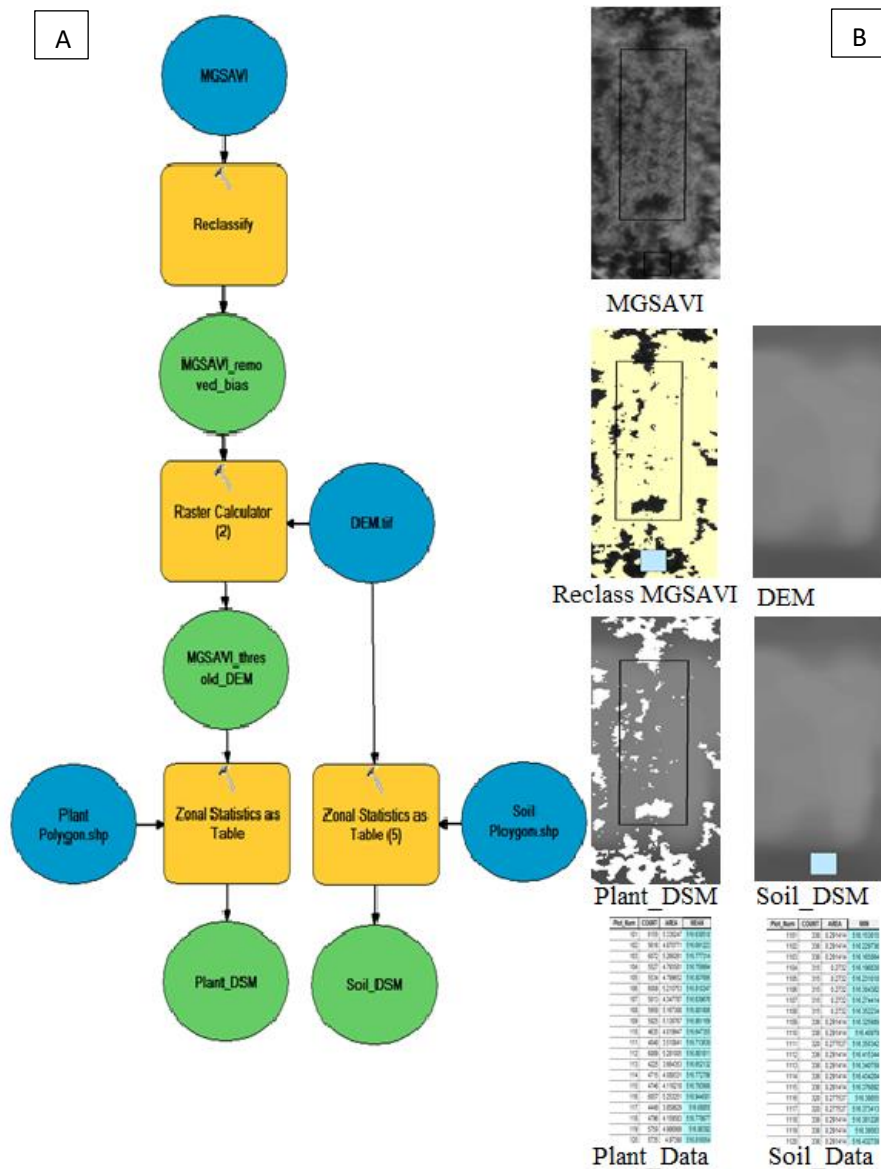
Figure 5.3. Workflow of canopy height extraction in ArcMap. A) Steps of image processing with the functions. 1. Use vegetative index threshold the image to derive the plant pixel mask. 2. Extract plant elevation from the DEM, plant polygons and plant mask 3. Extract soil elevation from the DEM and soil polygons 4. Calculate the normalized canopy height. B) The corresponding raster layers were derived from the workflow.

Derivation of lodging

Since canopy height was derived from the model, measures of the CHM at multiple dates were derived from 2019-site1, 2019-site2, and 2018-site1. Given the source of the vegetative index thresholds, DEM, and polygons, the model automatically derived all individual dates of CHM throughout the season. The percentage of height reduction

was calculated using the maximum heights in the peak and the minimum heights in the post-lodging utilizing the equation:

Height Reduction% = (Maximum CHM – Minimum CHM) / Maximum CHM *100

5.2.3 Statistical Analysis

Cross-validation and the General Linear Model in SAS Version 9.4 were used to validate the accuracy of the CHM for height estimation. Cross-validation was used to test the prediction accuracy of the model within a new dataset. A total of 320 plot results were resampled by year. The data from the 2019 sites (240 plots) and 2018 site (80 plots) were the training dataset and the validation dataset, respectively. Measured heights and image derived CHM from the same day in 2019 were analyzed using a linear regression model equation with CHM and measured height as the y and x variables. The CHM in 2018 was then imported into the equation to predict measured heights. The predicted heights were compared with the actual heights using the Root Mean Square Error (RMSE) and R-square. The COVTEST option tested the significance of the covariance. Residual options in PROC Mixed and the Univariate Statement were used to remove outliers, which *t*-distribution was over 3, and test the residuals for normality.

The maximum CHM throughout the season was compared with the maximum measured plant length in all plots to determine if images could estimate the plant length. The current study also measured the RMSE and r^2 between maximum height and harvest yield, using the same methodology as the previous comparison for height.

To evaluate the lodging assessments from the height reduction model, analysis of variance (ANOVA) was conducted in PROC MIXED to determine the percentage of height reduction response to fixed effects, including mixing ratio, variety, day of imaging, and their interaction. The model also assigned the site-years and their interaction with main effects as random. LSD was used to separate means at p-value <0.05. The lodging index from the ground measurements was analyzed in the same way.

5.3 Results

Accuracy of the CHM for Height Estimation

Comparing canopy heights taken on the same day by image-derived and ground measurements from the 2018 trial showed the image estimation to be $r^2 = 0.8839$ (Figure 5.4.a). The RMSE found the difference between the two measurements to be 2.6 cm. The plant length estimations (Figure 5.4.b) also found that applying the plot-maximum CHM over the season positively correlated with plant length ($r^2=0.807$ RMSE=4.63cm). These results demonstrate that crop heights can be extracted from UAV images, and CHM can represent canopy height when evaluating lodging severity. Further, the image-derived maximum heights were applied to predict pea yield. The yield prediction showed the maximum CHM did correlate with yield ($r^2=0.506$ RMSE=160 kg/ha) and could be a secondary trait for yield prediction.
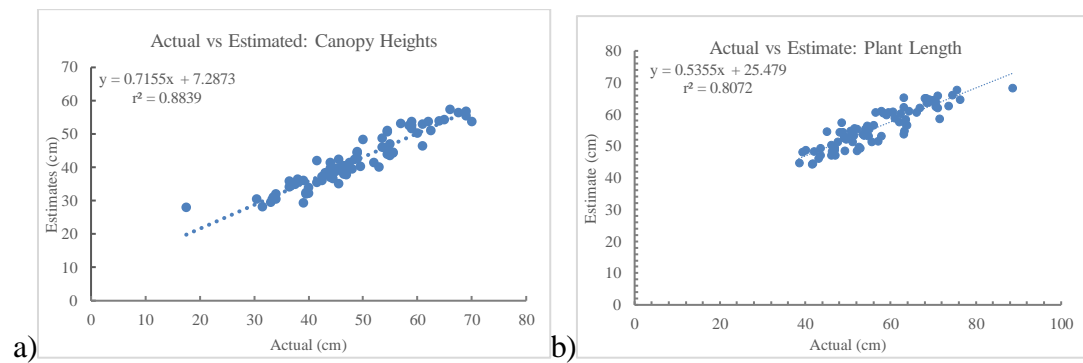
Figure 5.4: Validation of the image-derived heights to the ground measured heights in the testing dataset. a) Correlation between actual canopy heights and image-derived canopy heights taken on the same date b) Correlation between actual plant heights and maximum heights

Height reduction of lodging in pea

The image canopy heights were extracted in each imaged flight in each site-year (Appendix). Pea in 2019 exhibited lodging, where the canopy increased to a peak height and then declined. In comparison, pea in 2018 had a relatively stable height due to drought conditions and shorter plants. Treatments differed in maximum height and rate of height reduction, which corresponded to lodging severity.

Comparing the pea lodging index by ground and image-derived measurements

The UAV-imagery resulted in greater lodging differentiations than did the ground measurements for lodging assessment. The study analyzed the percentage of height reduction from the image estimation and reported significant variety and mixing ratio effects (Variety: $P=0.0126$; Semi-leafless proportion $P=0.0016$). In comparison, the results of the measured lodging index showed that the effects of the mixing were significant (Ratio: $P=0.0079$) and the non-significant variety effect ($P=0.0654$). Trends found that the height reduction illustrated more significant treatment lodging differentiations (Figure 5.5 and Figure 5.6). In the ratio results, the image-derived height reduction showed that the semi-leafless proportion over 83% was significantly lower than the semi-leafless proportion below 50%. In contrast, the lodging index by ground measurement showed that only the pure semi-leafless treatment differed from other ratios. This likely occurred due to ground measures being collected only once for the lodging whereas the plant and canopy height was subsampled from each plot. In comparison, the image estimation collected the height variation from early crop growth until harvest, with multiple flights measuring the entire plot.
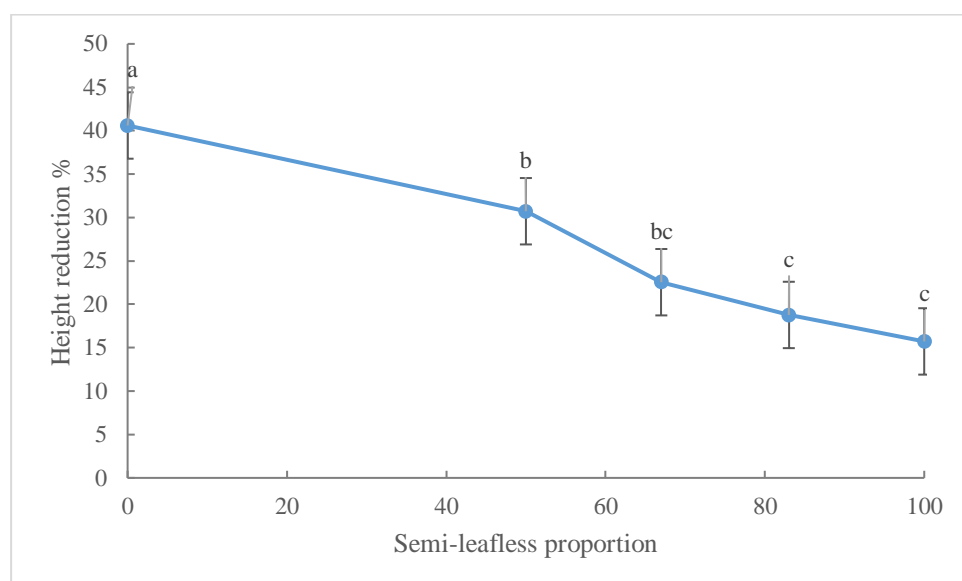
Figure 5.5: Height reduction by image estimation on leafed: semi-leafless pea ratio based on the combined varieties. The Mean of three site-years, one in 2018, and two in 2019. Means with different letters are significantly different. The error bar represents the positive standard error in $LSD_{0.05}$.
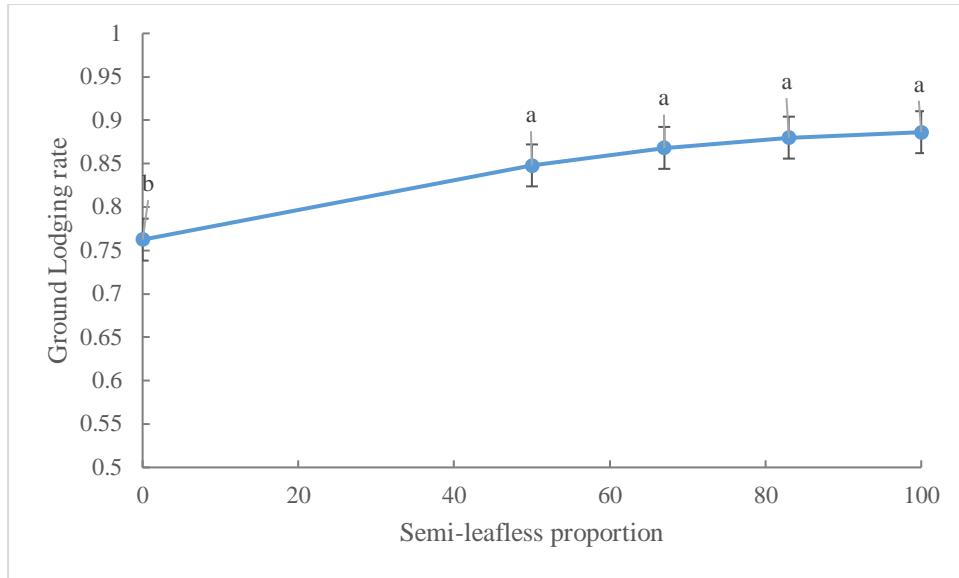
Figure 5.6: The lodging index by ground measurements on the leafed: semi-leafless pea ratio based on the combined varieties. Mean of three site-years, one in 2018, and two in 2019. Means with different letters are significantly different. The error bar represents the positive standard error in $LSD_{0.05}$.

5.4 Discussion

The CHM derived from UAV-multispectral image and DEM was able to estimate the canopy height of pea in field experiments across diverse environments and for many growth stages (r^2=0.88; Figure 5.4a). The CHM-derived height was extracted throughout the season at each site year, and the change of height indicated lodging severity.

Previous studies have used the DTM method, where the DSM is subtracted from DTM to capture canopy height (Sarkar et al., 2020, Alessandro et al., 2016). However, the UAV-DTM method needs to be improved for small-plot experimentation. The DTM was created through point cloud classification by smoothing non-terrain point clouds representing elevated ground features in the software. However, the point clouds are not definitively classified in the automatic classification. The software may classify the low plants as disabled points that lead to artifacts inside the DTM, and height to be inconsistently underestimated (Pixed4D support). Moreover, the CHM would also be overestimated if using the DTM method, particularly in flat topography. The DTM generation in Pix4D failed to represent sharp changes in the terrain (Pix4D support), which reduced the spatial variation among plots. In the present study, vegetative indices

and polygons were used to classify the plant terrain points and soil non-terrain points for each experimental plot. Then, the plant and soil elevation was measured in the DEM directly. Without the DTM, height estimation was enhanced, and the elevation among multiple date measurements matched.

Image-derived height measurements improved the canopy monitoring in three ways. First, it showed a canopy height in the pea crop over time. The current study used the CHM from BBCH 51 to BBCH 89 to estimate lodging. Likewise, Jiang et al. (2018) used image-derived heights from plant emergence through to reproductive stages to calculate the growth rate in maize. The second improvement was the maximum plant height, which indicated when lodging occurred. The current study also found that lodging related to final yield. Third, it improved the lodging assessment overall. Ground measurements are labor-intensive. Thus, the current study measured only five plant heights in a plot and only two measurements were taken during the season. In contrast, image-derived measurement was robotic, it measured pea height in the whole plot multiple times, which led to the image-derived lodging index being faster and more precise than manual measurements.

Three improvements will enhance the current study for estimation efficiency and accuracy. The first is plot annotation. The soil and plant polygons were manually drawn and adjusted for correction, and the soil polygons were set in front of the plot, therefore not covering all surrounding areas. Future research has started for automatic annotation of the whole soil area. The second is the image resolution. Previous studies have reported robust height estimation from UAV images in wheat (r^2=0.99) (Jimenez-Bern et al., 2018) and peanut (r^2=0.95) (Sarkar et al., 2020). Jimenez-Berni et al. (2018) used LiDAR sensors on a mobile ground platform and developed the 0.15 cm/pixel resolution. Sarkar et al. (2020) used an RGB camera with 0.49 cm/pixel resolution. In comparison, the current study's image resolution was 2.96 cm/pixels. The relatively low resolution resulted in less image processing power and was more time consuming. The third is calibration of point clouds. In the default setting, DEM, DSM, and CHM are meter (m) readings. However, the CHM results were underestimated compared to the measured heights in the calibration. A possible explanation was that ground measurements mostly measured the top of the canopy, while the calibration may smooth a part of points clouds representing the top canopy. Concerted efforts are needed to optimize the calibration of point clouds.

5.5. Conclusion

Canopy height of pea was estimated using digital elevation maps from UAV multispectral imagery. The current study extracted the CHM from images and modeled the canopy height variation due to lodging. The variation showed the maximum canopy height, indicated the timing of lodging, and evaluated the lodging severity. The current study also compared the lodging assessments from the image-derived estimation with the ground measurements. The results showed that the image estimation was highly correlated with the ground measured on canopy height measurement, but found greater significant differences in lodging between treatments indicating greater precision. This improvement is due to the UAV-imaging collecting heights in the overall plot consisting of multiple measurements over time. Furthermore, the height estimation methodology is effective not only in pulse crops, but is also advantageous in other crops, once the vegetative layer and the DEM of the objects are developed. As a phenotypic trait, resulting from the interaction of genetics and environment, canopy height measurement is also valuable in predicting growth rate and plant health. Traditional visual rating capture one data type at a time whereas UAV imaging, in the future, could robotically measure nitrogen level, plant moisture, canopy height, and many more traits simultaneously collecting continuous plant information from crop emergence through to maturity.

6. General Discussion and Conclusion

The current study tested the general hypothesis that blending semi-leafless and leafed pea will produce a yield increase compared to their monoculture by improving light interception and lodging resistance. The objectives of the first experiment were to compare the yield and agronomic performance of near-isogenic leafed and related semi-leafless blends as well as determine the effect of the varietal mixture on leaf-type blend for lodging, disease resistance, yield, and yield stability. A second experiment was to develop an optimal blend targeting higher yield and improved agronomics. A third experiment was to quantify lodging severity using DEM from multispectral images to evaluate the most lodging-resistant blend.

Both the first and second experiments compared the near-isogenic leaf-type blends with the monocultures in several mixing ratios. The solar radiation interception was measured during early vegetation to determine canopy closure. The results showed that the canopy of leaf type blends had a similar closure rate and light interception at full canopy coverage (Table 4.2; Table 3.6). Harvey (1972) found the photo-assimilation of CO_2 per unit area and unit dry weight to be comparable in semi-leafless and leafed pea. Although the photo-assimilation of CO_2 per unit dry weight by the semi-leafless crop was only 18% of the mean value for the leafed varieties, the tendrils of the semi-leafless type have a higher proportion of photosynthetically inert tissue than does the leafed type leaflets. The authors concluded that the tendrils are photosynthetically comparable to the leaflets. Kof et al. (2004) measured the leaf shaded area on the soil surface at the third sub-apical leaf stage of leafed and semi-leafless pea, and found that the leafed pea had a greater photo-assimilation area. However, the author concluded that the high amount of leaf shading in the leafed canopy would lead to lower leaves having a reduced chlorophyll content. Although the semi-leafless pea reduced leaflet and light interception, the extended tendrils and stipules, which accumulate extra Chlorophyll a + b, as well as their additional lower tendrils and stipules increases the photo-assimilation area. The productivity of plants depends on their assimilation area and chlorophyll content (Nichiporovich et al., 1977; Kof et al., 2004). In the present study, the above-ground biomass was generally no different among leaf types (Table 3.6; Table 4.2). It is worthwhile to note that the photo-assimilation area and chlorophyll content among leafed pea, semi-leafless pea, and leaf-type blends have a similar plant productivity at the 88 plants/ m^2 seeding rate.

The leaf-type blend prevented yield loss of the leafed pea due to lodging, producing a higher overall yield than the leafed monoculture. The present study calculated the pea height reduction from lodging using UAV-imagery. The time-series height variation showed a trend where the leafed component in the leaf-type blend would aggravate lodging. Both the ground lodging height index and image-derived height index determined that lodging severity would be significantly increased if the leafed proportion was over 33%. When comparing the lodging severity among the different leaf types and the leaf-type blends, the height reduction of the sole semi-leafless type was the lowest. It can be concluded that the semi-leafless monoculture has the greatest lodging resistance when compared to the leafed monoculture and the leaf-type blend. The high lodging resistance of semi-leafless pea has also been shown by Syrovy (2014), in which the semi-leafless monoculture remained upright during the reproductive and maturation stages.

The yield results of leaf types corresponded to secondary field traits, particularly lodging. Results showed that the leaf-type blend had agronomic and yield benefits compared to the leafed pea but no difference compared to the semi-leafless pea. In the first experiment, the 75:25 SL/L blend was found to have a 11% lodging decrease and a 5% disease severity reduction compared with the leafed monoculture. However, this did not result in higher yield than the leafed monoculture. In comparison, the semi-leafless monoculture had the greatest lodging resistance across varieties and a 13% significant higher yield than the leafed monoculture in CDC Centennial. While the blends showed statistically similar yield, the semi-leafless monoculture had significantly lower lodging and greater yield than the leaf blend. In the second experiment, the estimated optimal 86:14% blend had similar yield compared to the semi-leafless monoculture, which in turn had a 13% yield increase over the leafed monoculture.

Several studies have reported yield benefits of leaf-type blends compared to sole monocultures. The most significant yield increase was the blend grown in a 75:25% SL/L ratio in an organic cropping system (Syrovy et al., 2015). As was discussed in Chapter 1 and 2, the yield difference between Syrovy's study and the present study is likely due to the difference in weed competition between the conventional and organic systems. Other studies conducted in the conventional system found a significant yield increase (Cupina et al., 2010; Antanasovic et al., 2011; Schouls & Langelaan, 1994). However, the yield increases were not reliable as these experiments were conducted

using varied genotypes in the leaf-type blend. The varying genotypes did not only differ in leaf types but also in other traits such as height, yield potential, disease resistance, and lodging. Antanasovic et al. (2011) found that the 75:25% ratio provided a 9 and 39% yield increase compared to a pure semi-leafless and leafed stand. Živanov et al. (2019) evaluated a 50:50 mixing ratio and reported the relative mixture yield of the leafed and semi-leafless components to have a positive LER (1.03 and 1.06), in which the leafed pea in the mixture developed more pods and seeds per pod compared to the sole leafed pea under drought conditions. Also, under more favorable weather conditions, the leafed pea had the highest yield compared to the semi-leafless pea and leaf-type blends. However, Gollner et al. (2019) conducted the blends in the 50:50% mixing ratio with varied varieties and reported no important yield increase. Therefore, this study using the near-isogenic pairs, provided a more reliable result to determine whether blending leafed and semi-leafless peas could provide a consistent agronomic and yield improvement.

The results of this study showed that the near-isogenic leafed pea did not overcome the related semi-leafless pea in lodging, disease, canopy light interception or biomass. Lowering the leafed pea portion ratio is unable to significantly improve the performance of the overall leaf-type blend. This can explain why the 86:14 blend had similar lodging as the semi-leafless pea and resulted in no yield difference. It can be concluded that while the leafed and semi-leafless pea only differ in leaf type, blending the two types can reduce leafed lodging and prevent yield loss, but does not increase the overall yield over the semi-leafless monoculture.

The overall objective of the project was to develop a leaf blend mixture that would have a higher yield than the current semi-leafless pea grown alone. To help achieve this the experiment was conducted using high yielding elite lines. Chapter 1 found that the varietal mixture did not improve the pea leaf-type blends by enhancing agronomic traits, yield, and yield stability. Yield results only showed a significant difference by genotype. The RDis results saw a potential disease reduction by mixing susceptible varieties with a moderately susceptible variety. An exhaustive yield comparison has also been conducted among the non-isogenic, near-isogenic leaf blends and monocultures. The results showed that the yield of the leaf-type blend was not different regardless of near-isogenic or non-isogenic blends; they were generally intermediate to the monoculture regardless of the blending genotype backgrounds (Table 3.7).

This thesis project evaluated four pairs of near-isogenic lines in the two experiments,

for a total of eleven site years, and determined that leaf-type blends have no significant yield and agronomic benefits compared to the current semi-leafless pea production. Leafed pea is no longer being bred for food use in Western Canada and hasn't been for decades; whereas semi-leafless breeding has been a great success and many elite varieties have been released. Currently, the semi-leafless varieties have better agronomic performance than the leafed varieties for many traits (i.e., lodging, market class, yield stability, etc.) and a similar yield potential as the optimal leaf-type blend. Therefore the author, after examining the project results, recommends that leaf-type blending is unwarranted for field application.

7. APPENDIX

7.1 Covariance of Variation of Site Years

Results showed the disease severity in 19-blend-three sites had a significant variation, where CV>30% is considered as an inconsistent measurement in agriculture (Kwanchai, 1984). Other response variables were <30% and results were acceptable.

Table 7.1 Coefficient of Variance for the experiment one variable at 5 site-year during 2018 &2019 in central Saskatchewan.

	Coefficient of Variance [%]				
	Site-Years				
Response Variable	18-blend-one	18-blend-two	19-blend-one	19-blend-two	19-blend-three
Yield	20.31	12.52	9.07	9.80	15.33
Biomass	16.96	18.63	13.88	11.85	18.03
Disease	17.35	16.33	NA	19.97	**32.63**
Lodging height index	14.25	12.29	15.56	11.58	12.61
Maturity	3.10	3.80	NA	2.06	1.37

Table 7.2 Coefficient of Variance for the response variables at 5 site-year during 2017& 2018 &2019 in central Saskatchewan.

	Coefficient of Variance [%]					
	17-Ratio-One	18-Ratio-One	18-Ratio-Two	19-Ratio-One	19-Ratio-Two	19-Ratio-Three
Source of Variation						
Yield	10.88	21.12	18.40	15.54	10.81	**31.20**
Biomass	14.32	17.36	18.82	18.55	15.74	22.81
Disease	NA	17.19	17.00	NA	**31.92**	16.69
Lodging height index	NA	16.19	10.74	9.96	12.56	16.26

7.2 The Temporal Variation of Image-derived Canopy Height at Site Years.
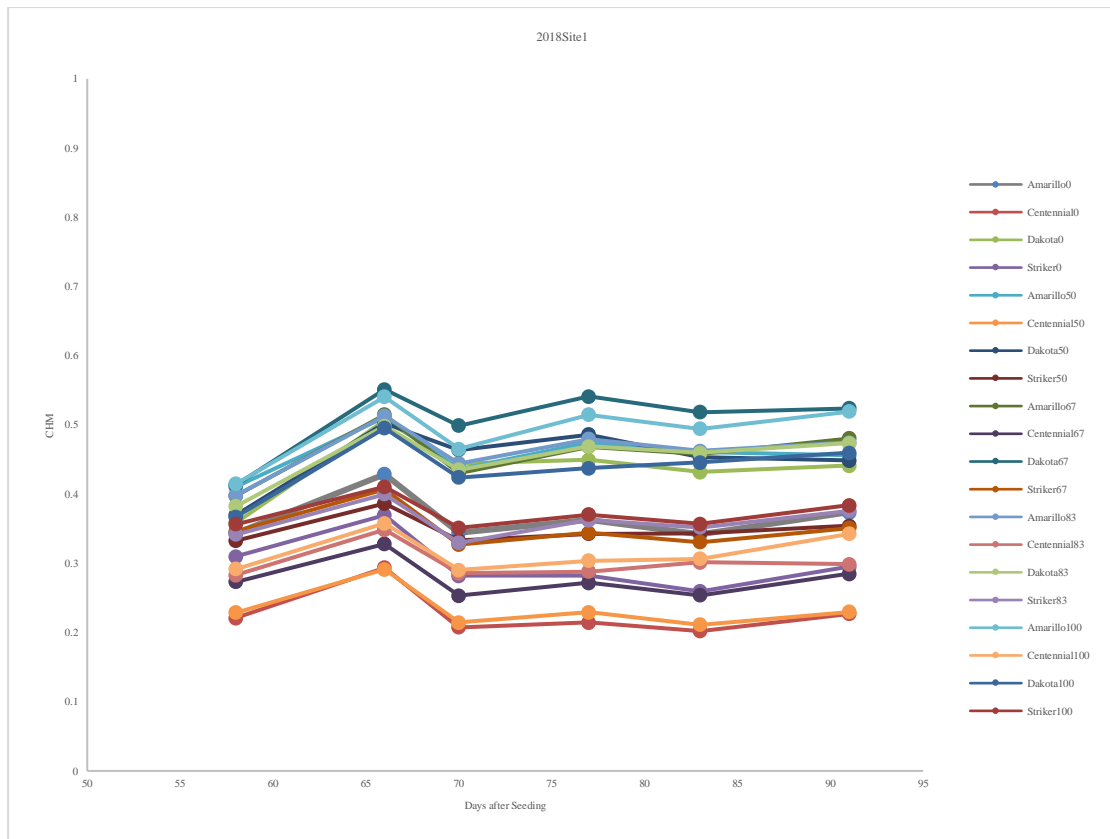

2019Site 1
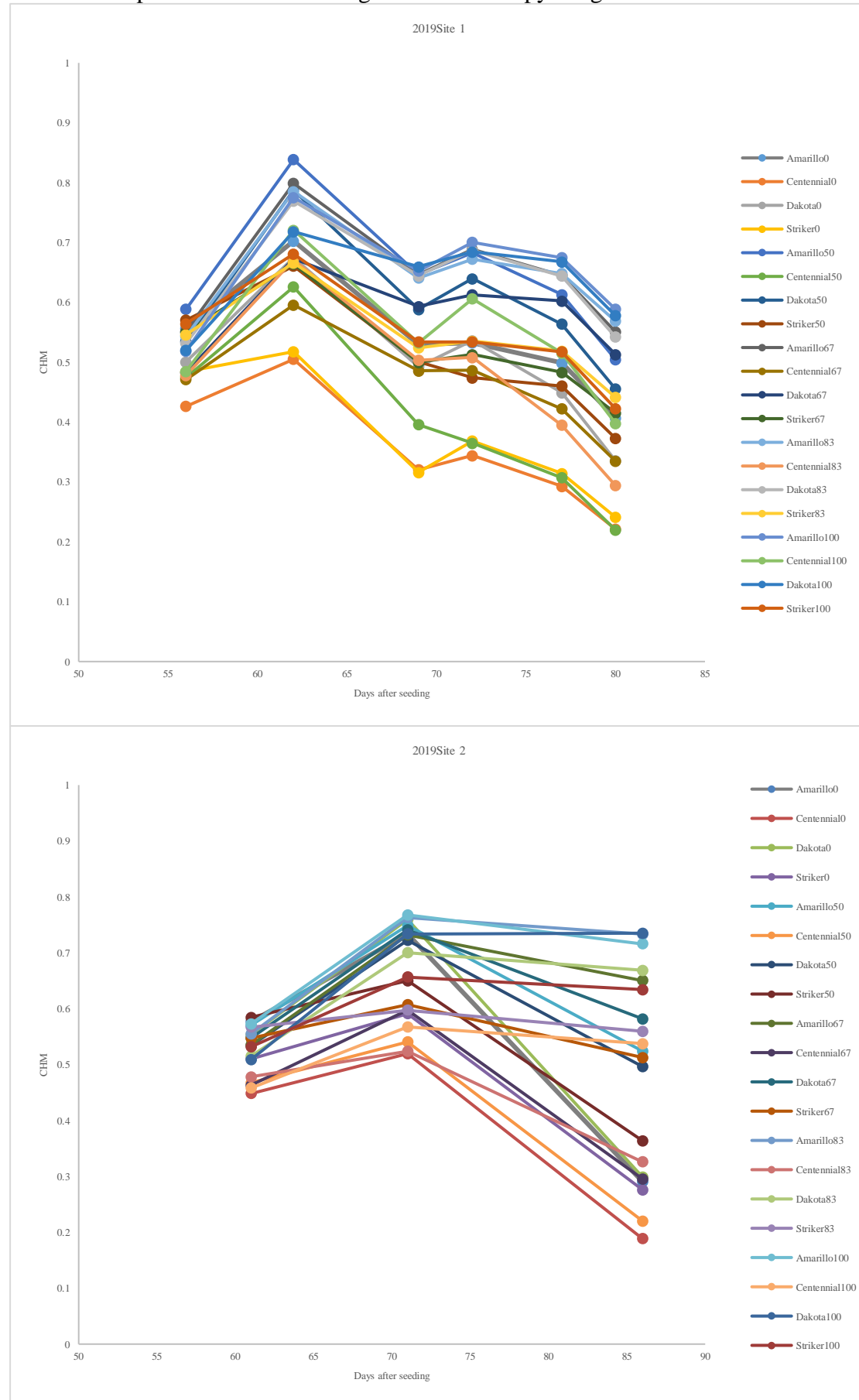
2019Site 2

Figure 7.1: The mean of image-derived heights of treatments in each flight from pre-lodging to post-lodging in 2018 and 2019 sites.

8. Reference

Alessandro, M., Salvatore, D.G., & Andrea, B. (2016). Assessment of a canopy height model (CHM) in a vineyard using UAV-based multispectral imaging. *International Journal of Remote Sensing*, 38(8-10), 1-11. https://doi.org/10.1080/01431161.2016.1226002.

Amelin, A.V., Parakhin, N.V. (2003). Scientific grounds for pea selection for fodder production. *Kormoproizvodstvo*, 2, 20-25.

Antanasovic. S., Mikic, A., Cupina. B., Krstic. D., Mihailovic. V., Eric. P., & Milosevic. B., (2011). Some agronomic aspects of the intercrops of semi-leafless and normal-leafed dry pea cultivars. *Pisum Genetic*, 43, 25-28.

Arisnabarreta, S., Miralles, D. (2008). Radiation effects on potential number of grains per spike and biomass partitioning in two- and six-rowed near isogenic barley lines. *Field Crops Research*, 107(3), 203-210. https://doi.org/10.1016/j.fcr.2008.01.005.

Banniza, S., Hashemi, P., Warkentin, T., Vandenberg, A., & Davis, A. R. (2011). The relationships among lodging, stem anatomy, degree of lignification, and resistance to mycosphaerella blight in field pea (*Pisum sativum*). *Canadian Journal of Botany*, 83, https://doi.org/954-967. 10.1139/b05-044.

Beeck, C.P., Wroth, J., & Cowling, W.A. (2006). Genetic variation in stem strength in field pea (*Pisum sativum* L.) and its association with compressed stem thickness. *Australian Journal of Agricultural Research*, 57(2), 193-199. https://doi.org/10.1071/AR05210.

Bilgili, U., Uzun, A., Sincik, M., Yavuz, M., Aydınoğlu, B., Çakmakçı, S., Geren, H., Avcıoglu, R., Nizam, İ., Tekeli, A.S., Gül, İ., Anlarsal, A.E., Yücel, C., Avcı, M., Acar, Z., Ayan, İ., Üstün, A., & Açıkgöz, E., (2010). Forage yield and lodging traits in peas (*Pisum sativum* L.) with different leaf types. *Turkish Journal of Field Crops,* 15(1), 50-53.

Blanco A.R., Simeone, A., & Gadaleta. (2006). Detection of QTLs for grain protein content in durum wheat. *Theoretical and Applied Genetics,* 112: 1195–1204 https://doi.org/10.1007/s00122-006-0221-6

Brouwer, D.J., St Clair, D.A. (2004). Fine mapping of three quantitative trait loci for late blight resistance in tomato using near isogenic lines (NILs) and sub-NILs. *Theoretical and Applied Genetics*, 108(4), 628-638. https://doi.org/10.1007/s00122-003-1469-8.

Bueckert. R. A., Wagenhoffer. S., Hnatowich. G., & Warkentin, T.(2014). Effect of heat and precipitation on pea yield and reproductive performance in the field. *Canadian Journal of Plant Science,* 95(4), 629-639. https://doi.org/10.4141/cjps-2014-342 *Canadian Journal of Plant Science*, 61(2), 463-465. https://doi.org/10.4141/cjps81-063

Carter, P.R., Hudelson, K.D. (1988). Influence of simulated wind lodging on corn growth and grain yield. *Journal of Production Agriculture,* 1(4), 295-299. https://doi.org/10.2134/jpa1988.0295

Chauhan S., Darvishzadeh, R., van-Delden, S.H., Boschetti, M., Nelson, A. (2021). Mapping of wheat lodging susceptibility with synthetic aperture radar data, *Remote Sensing of Environment*, 259, https://doi.org/10.1016/j.rse.2021.112427.

Chiang, K., Liu, S., Bock, C. H., and Gottwald, T. R. (2014). What interval characteristics make a good categorical disease assessment scale?. Analytical and *Theoretical Plant Pathology*, 104(6), 575-585. https://doi.org/10.1094/PHYTO-10-13-0279-R

Chu, T. X., Starek, M. J., Brewer, M. J., Murray, S. C., and Pruter, L. S. (2017). Assessing lodging severity over an experimental maize (*Zea mays* L.) Field Using UAS Images. *Remote Sensing*, 9(9), 923. https://doi.org/10.3390/rs9090923

Cowger, C., Mundt, C.C. (2002). Effects of wheat cultivar mixtures on epidemic progression of Septoria tritici blotch and pathogenicity of Mycosphaerella graminicola. *Phytopathology*, 92(6), 617-623. https://doi.org/10.1094/PHYTO.2002.92.6.617

Creissen, H.E., Jorgensen T.H., & Brown J.K.M. (2016). Increased yield stability of field-grown winter barley (*Hordeum vulgare* L.) varietal mixtures through ecological processes, *Crop Protection*, 85,1-8. https://doi.org/10.1016/j.cropro.2016.03.001.

Cupina, B., Krstic, Đ., Antanasovic, S., Eric, P., Pejic, B., Mikic, A., & Mihailovic, V. (2010). Potential of the intercrops of normal-leafed and semi-leafless pea cultivars for forage production. *Pisum Genetics,* 42: 11-14.

Darras, S., McKenzie R.H., Olson, M.A., & Willenborg C.J., (2014). Influence of genotypic mixtures on field pea yield and competitive ability. *Canadian Journal of Plant Science.* 95(2), 315-324. https://doi.org/10.4141/cjps-2014-253

Villegas-Fernández, Á. M., Amarna, A., Moral, J., & Rubiales, D. (2021). Crop Diversification to Control Powdery Mildew in Pea. *Agronomy*, *11*(4), 690. https://doi.org/10.3390/agronomy11040690

Finckh, M., Gacek, E., Goyeau, H., Lannou, C., Merz, U. Mundt. C., Munk, L., Nadziak, J., Newton, A., & Vallavieille-Pope, C.D. (2000). Cereal variety and species mixtures in practice, with emphasis on disease resistance. *Agronomie*, EDP Sciences, 20 (7), 813-837. https://doi.org/10.1051/agro:2000177

FoodData Center. (2019). *Peas, green, raw.* Retrieved from https://fdc.nal.usda.gov/fdc-app.html#/food-details/170419/nutrients.
forage type pea lines of contrasting leaf type. European Journal of Agronomy. 22(1), 85-94.

Fréville, H., Roumet, P., Rode, N. O., Rocher, A., Latreille, M., Muller, M. H., & David, J. (2019). Preferential helping to relatives: A potential mechanism responsible for lower

yield of crop variety mixtures?. *Evolutionary applications*, 12 (9), 1837-1849. https://doi.org/10.1111/eva.12842

Fumiki, H., Kenji O., (2012). Estimation of vertical plant area density profiles in a rice canopy at different growth stages by high-resolution portable scanning lidar with a lightweight mirror, *ISPRS Journal of Photogrammetry and Remote Sensing*, 74, 11-19. https://doi.org/10.1016/j.isprsjprs.2012.08.001.

Lafond, L G., Evans, E. & Ali-khan, S. T. (1981). Comparison of near-isogenic leafed, leafless, semi-leafless, and reduced stipule lines of peas for yield and associated traits.

Gitelson, A.A., Kaufman. J.Y., Merzlyak, N.M. (1996). Use of a green channel in remote sensing of global vegetation from EOS-MODIS, *Remote Sensing of Environment*, 58(3), 289-298. https://doi.org/10.1016/S0034-4257(96)00072-7.

Goldenberg, J. b. (1965). "Afila", a New Mutation in Pea (*Pisum Sativum*. L.). *Boletin Genetico*, 1, 27–31.

Goldman, I. L., & Gritton, E. T. (1992). Seasonal variation in leaf component allocation in normal, afila, and afila-tendrilled acacia pea foliage near-isolines. *Journal of the American Society for Horticultural Science*, 117(6), 1017-1020. https://doi.org/10.21273/JASHS.117.6.1017

Gollner, G., Starz, W., Friedel, J.K. (2019). Crop performance, biological N fixation and pre-crop effect of pea ideotypes in an organic farming system. *Nutrient Cycling in Agroecosystems*, 115(3), 391–405. https://doi.org/10.1007/s10705-019-10021-4

Gourlay, W.C, Hofer, M.J, & Ellis, N.T.H (2000). Pea Compound Leaf Architecture Is Regulated by Interactions among the Genes *UNIFOLIATA, COCHLEATA, AFILA*, and *TENDRIL-LESS*. *Plant Cell*. 12(8). 1279-1295. https://doi.org/10.1105/tpc.12.8.1279.

Government of Saskatchewan. (2017). *Dry pea.* Retrieved from http://publications.gov.sk.ca/documents/20/86385-dry%20pea.pdf

Grains Research Development Corporation. (2017). *GRDC Grow notes-Field Peas.* Retrieved from https://grdc.com.au/resources-and-publications/grownotes/crop-agronomy/field-pea-southern-region-grownotes

Hagem, V., Philippe, D., & Bateson, W. (1911). A case of gametic coupling in Pisum. *Z.Ver-erbungslehre* 6, 248. https://doi.org/10.1007/BF01778375

Harker, K. N., Blackshaw, R.E., & Clayton, G.W. (2001). Timing Weed Removal in Field Pea (*Pisum sativum*). *Weed Technology*, 15(2), 277-283.

Harker, K. N., Clayton. G. W., & Blackshaw. R. E. (2008). Comparison of leafy and semi-leafless pea for integrated weed management. *Weed Technology*, 22(1), 124–131. https://doi.org/10.1614/WT-07-090.1

Harvey, D. M. (1972). Carbon dioxide photoassimilation in normal-leaved and mutant forms of Pisum sativum L. *Annals of Botany*, 36(5), 981-991. https://doi.org/10.1093/oxfordjournals.aob.a084659

Harvey, D. M., (1978). The photosynthetic and respiratory potential of the fruit in relation to seed yield of leafless and semi-leafless mutants of *Pisum sativum* L. *Annals of Botany*, 42, 331-336.

Harvey, D. M., & Goodwin, J., (1978). The photosynthetic net carbon dioxide exchange potential in conventional and 'leafless' phenotypes of *Pisum safivum* L. in relation to foliage area, dry matter production and seed yield. *Annals of Botany,* 42, 1091-1098.

Heath, M.C., Hebblethwaite, P.D. (1985). Solar radiation interception by leafless, semi-leafless and leafed peas (*Pisum sativum*) under contrasting field conditions. *Annals of Applied Biology*, 107, 309-318. https://doi.org/10.1111/j.1744-7348.1985.tb01575.

Holman, F.H., Riche, A B., Michalski, A., Castle M., Wooster, M.J., & Hawkesford, M.J. (2016). High Throughput Field Phenotyping of Wheat Plant Height and Growth Rate in Field Plot Trials Using UAV Based Remote Sensing. *Remote Sensing*, 8(12),10-31. https://doi.org/10.3390/rs8121031

Hu, X., Sun, L., Gu, X., Sun, Q., Wei, Z., Pan, Y., & Chen, L. (2021). Assessing the Self-Recovery Ability of Maize after Lodging Using UAV-LiDAR Data. *Remote Sensing*, 13(12), 2270. https://doi.org/10.3390/rs13122270

Jackson, L. F., & Wennig, R. W. (1997). Use of wheat cultivar blends to improve grain yield and quality and reduce disease and lodging. *Field Crops Research*, 52(3), 261-269. https://doi.org/10.1016/S0378-4290(97)00007-5

Jensen, E.S. (1996). Grain yield, symbiotic N2 fixation and interspecific competition for inorganic N in pea-barley intercrops. *Plant and Soil*, 182, 25–38. https://doi.org/10.1007/BF00010992

Jiang, Y, Li, C, Paterson, A.H., Sun, S, Xu, R., & Robertson, J. (2018). Quantitative Analysis of Cotton Canopy Size in Field Conditions Using a Consumer-Grade RGB-D Camera. Frontiers of Plant Science, 8, 2233. https://doi.org/10.3389/fpls.2017.02233

Jiang, Y., Li, C., Paterson, H. A. (2018). High throughput phenotyping of cotton plant height using depth images under field conditions. Computers and Electronics in Agriculture, 130, 57-68, https://doi.org/10.1016/j.compag.2016.09.017.

Jimenez-Berni, J.A, Deery, D.M., Rozas-Larraondo, P., Condon, A.G., Rebetzke G.J., James R.A., Bovill, W.D., Furbank, R.T., & Sirault, X.R.R.(2018). High Throughput Determination of Plant Height, Ground Cover, and Above-Ground Biomass in Wheat with LiDAR. Frontiers of Plant Science, 9,237. https://doi.org/10.3389/fpls.2018.00237

Jones, M.W., Boyd, E.C., & Redinbaugh, M.G. (2011). Responses of maize (*Zea mays* L.) near isogenic lines carrying *Wsm1, Wsm2,* and *Wsm3* to three viruses in the

Potyviridae. Theoretical and Applied Genetics, 123(5), 729–740. https://doi.org/10.1007/s00122-011-1622-8

Joseph, R.P., Gyozo, J. (2007). Digital Terrain Modelling. Heidelberg, German: Springer-Verlag Berlin Heidelberg

Kiær, L. P., Skovgaard, I. M., & Østergård, H. (2009). Grain yield increase in cereal variety mixtures: a meta-analysis of field trials. *Field Crops Research*, *114*(3), 361-373. https://doi.org/10.1016/j.fcr.2009.09.006

Klimek-Kopyra, A., Głąb, T., & Lorenc-Kozik A. (2015). Estimation of tendrils of parameters depending on the sowing methods, in contrasting *Pisum sativum* L. varieties. *Romanian Agricultural Research* 32.

Kristoffersen, R., Jørgensen L.N, Eriksen, L.B, Nielsen, G.C., & Kiær, L.P. (2020). Control of Septoria tritici blotch by winter wheat cultivar mixtures: Meta-analysis of 19 years of cultivar trials, Field Crops Research, 249 (1). https://doi.org/10.1016/j.fcr.2019.107696

Kujala, V. (1953). Federbse bei welcher die ganze Blattspreite in Ranken umgewandelt ist. Archivum, Societatis Zoologicae Botanicae Fennicae 'Vanamo' 8:44-45.

Lanning, S.P., Martin, J.M., Stougaard, R.N., Guillen-Portal, F.R., Blake, N.K., Sherman, J.D., Robbins, A.M., Kephart, K.D., Lamb, P., Carlson, G.R., Pumphrey, M. & Talbert, L.E. (2012), Evaluation of Near-Isogenic Lines for Three Height-Reducing Genes in Hard Red Spring Wheat. *Crop Science*, 52(3), 1145-1152. https://doi.org/10.2135/cropsci2011.11.0625

Le May. C., Ney, B., Lemarchand, E., Schoeny, A., & Tivoli, B. (2009). Effect of pea plant architecture on spatiotemporal epidemic development of ascochyta blight (*Mycosphaerella pinodes*) in the field. Plant Pathology, 58 (2), 332-343. https://doi.org/10.1111/j.1365-3059.2008.01947.x

Lecoeur. J., Bertrand, N. (2003). Change with time in potential radiation-use efficiency in field pea. *European Journal of Agronomy*, 19(1), 91-105. https://doi.org/10.1016/S1161-0301(02)00019-9.

Liu, T., Li, R., Zhong, X., Jiang, M., Jin, X., Zhou, P., Liu, S., Sun, C., Guo, W. (2018). Estimates of rice lodging using indices derived from UAV visible and thermal infrared images. *Agricultural and Forest Meteorology*, 252: 144-154. https://doi.org https://doi.org/10.1016/j.agrformet.2018.01.021.

Liu, Z., Li, C., Wang, Y., Huang, W., Ding, X., Zhou, B., Wu, B., Wang, D., Shi, J., (2012). Comparison of Spectral Indices and Principal Component Analysis for Differentiating Lodged Rice Crop from Normal Ones. *Computer and Computing Technologies in Agriculture*, 5:84-92. https://doi.org/10.1007/978-3-642-27278-3_10

Maiti, R.K., Rodriguez H.G., (2012), Research advances in vegetable crops, Puspa Publishing House. Retrieved from https://www.pphouse.org

Marx, B. D., Stroup, W.W.(1993). Analysis of spatial variability using PROC MIXED. Conference on *Applied Statistics in Agriculture*. https://doi.org/10.4148/2475-7772.1371

Mead, R., Willey, W. R. (1980) The Concept of Land Equivalent Ratio and Advantages in Yields from Intercropping. *Experimental Agriculture*, 16(3), 217-228.

Mikić, A., Mihailović, V., Ćupina, B., Kosev, V., Warkentin, T., Mcphee, K., Ambrose, M., Hofer, J., & Ellis, N. (2011). Genetic Background and Agronomic Value of Leaf Types in Pea (*Pisum sativum*). *Field and Vegetable Crops Research*, 48(2), 275-284. https://doi.org/10.5937/ratpov1102275M.

Miralles, D.J., Slafer, G.A. (1997). Radiation interception and radiation use efficiency of near-isogenic wheat lines with different height. Euphytica, 97, 201–208. https://doi.org/10.1023/A:1003061706059

Murakami, H., Mizuta, R., & Shindo, E. (2012). Future changes in tropical cyclone activity projected by multi-physics and multi-SST ensemble experiments using the 60-km-mesh MRI-AGCM. *Climate Dynamics*, 39:2569–2584. https://doi.org/10.1007/s00382-011-1223-x

Pelzer, E., Bazot, M., Makowski, D., Corre-Hellou, G., Naudin, C., Rifaï, M., Baranger, E., Bedoussac, L., Biarnès, V., Boucheny, P., Carrouée, B., Dorvillez, D., Foissy, D., Gaillard, B., Guichard, L., Mansard, M., Omon, B., Prieur, L. Yvergniaux, M., & Jeuffroy, M.H. (2012). Pea-wheat intercrops in low-input conditions combine high economic performances and low environmental impacts. *European Journal of Agronomy*. 40, 39-53. https://doi.org/10.1016/j.eja.2012.01.010.

Podgorska-Lesiak, M., & Sobkowicz, P. (2013). Prevention of pea lodging by intercropping barley with peas at different nitrogen fertilization levels. *Field Crops Research,* 149(1), 95-104. https://doi.org/10.1016/j.fcr.2013.04.023

Qin, X., Li, Y., Shi, C., Song, D., Wen, X., Liao, Y., & Siddique, K. H. (2019). The number of cultivars in varietal winter-wheat mixtures influence aboveground biomass and grain yield in North China. *Plant and Soil*, 439(1), 131-143. https://doi.org/10.1007/s11104-019-04084-z

Ren, Y., Setia, R., Warkentin, T. D., & Ai, Y. (2021). Functionality and starch digestibility of wrinkled and round pea flours of two different particle sizes. *Food Chemistry*, 336, 127711. https://doi.org/10.1016/j.foodchem.2020.127711

Sarkar, S., Cazenave, A.B, & Oakes, J. (2020). High-throughput measurement of peanut canopy height using digital surface models. *Plant Phenome Journal*, 3(1). https://doi.org/10.1002/ppj2.20003

Saskatchewan Pulse Grower. (2021). *Saskatchewan Variety Guide.* Retrieved from https://saskpulse.com/growing-pulses/peas/

Schouls, J., Landgelaan, J.G. (1994). Lodging and yield of dry peas (*Pisum sativum* L.) as influenced by various mixing ratios of a conventional and a semi-leafless cultivar.

Journal of Agronomy and Crop Science, 172(3), 207-214. https://doi.org/10.1111/j.1439-037X.1994.tb00168.x

Setter, T.L., Laureles, E.V., & Mazaredo, A.M. (1997). Lodging reduces yield of rice by self-shading and reductions in canopy photosynthesis, *Field Crops Research*, 49(2-3), 95-106. https://doi.org/10.1016/S0378-4290(96)01058-1.

Simmonds, N. W. (1962). Variability in crop plants, its use and conservation. *Biological Reviews*, *37*(3), 422-465. https://doi.org/10.1111/j.1469-185X.1962.tb01620.x

Singh, A., Srivastava, C. (2015). Effect of plant types on grain yield and lodging resistance in pea (*Pisum sativum* L.). *Indian Journal of Genetics and Plant Breeding*, 75(1). https://doi.org/10.5958/0975-6906.2015.00008.5.

Singh, A.K, Tyagi. N., & Srivastava, C.P. (2012). Interrelationship among components of lodging and yield in pea. *Vegetable Science* 39(2), 183-185.

Singh, D., Wang, X., Kumar, U., Gao, L., Noor, M., Imtiaz, M., Singh, R.P., & Poland, J. (2019). High-Throughput Phenotyping Enabled Genetic Dissection of Crop Lodging in Wheat. *Frontiers of Plant Science*. 10, 394. https://doi.org/10.3389/fpls.2019.00394

Smitchger, J. (2017). Quantitative trait loci associated with lodging, stem strength, yield, and other important agronomic traits in dry field peas. *Biology*.

Smitchger, J., Weeden, N., Akin, I., & Warkentin, T. (2020) Stress equation for a cantilever beam: a model of lodging resistance in field pea. *International Agrophysics*, 34, 213-222. https://doi: 10.31545/intagr/118318.

Smithson, J.B., Lenne, J.M. (1996). Varietal mixtures: a viable strategy for sustainable productivity in subsistence agriculture. *Annual of Applied Biology*, 128(1), 127-158. https://doi.org/10.1111/j.1744-7348.1996.tb07096.x

Spies, J. M., Warkentin, T., & Shirtliffe, S. J., (2010). Variation in field pea (*Pisum sativum*) cultivars for semi-leafless al branching and weed competition. *Weed Science,* 59(2), 218-223. https://doi.org/10.1614/WS-D-10-00079.1

Stam, P., Zeven, A.C. (1981). The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing. *Euphytica*, 30, 227–238. https://doi.org/10.1007/BF00033982

Statista, (2018). *Production volume of field peas worldwide from 2013 to 2017, by country*. Retrieved from https://www.statista.com/statistics/722160/field-peas-production-volume-by-country-worldwide

Stelling, D. (1989). Problems of Breeding for Improved Standing Ability in Dried Peas (*Pisum sativum* L.). *Journal of Agronomy and Crop Science*, 163(1), 21-32. https://doi.org/10.1111/j.1439-037X.1989.tb00733.

Stoloff, P.H. (1970). Correcting for Heterogeneity of Covariance for Repeated Measures Designs of the Analysis of Variance. *Western Journal of Nursing Research*, 30, 909-924. https://doi.org/10.1177/019394599201400111

Syrovy, L (2014). *Effect and Underlying Mechanisms of Cultivar Mixtures on Weed and Disease Suppression in Field Pea (Pisum sativum).* (Master's thesis, University of Saskatchewan, Saskatoon, Canada). Retrieved from https://harvest.usask.ca/handle/10388/ETD-2014-02-1450

Syrovy, L. D., Banniza, S., & Shirtliffe, S.J. (2015). Yield and agronomic advantages of pea leaf type mixtures under organic management. *Agronomy Journal*, 107(1), 113-120. https://doi.org/10.2134/agronj14.0218

Trenbath, B. R. (1974). Biomass productivity of mixtures. In Advances in agronomy (Vol. 26, pp. 177-210). Academic Press. https://doi.org/10.1016/S0065-2113(08)60871-8

Vasilakoglou, I., Dhima, K., Anastassopoulos, E., Lithourgidis, A, Gougoulias, N., & Chouliaras, N. (2011). Oregano green manure for weed suppression in sustainable cotton and corn fields. *Weed Biology and Management*, 11(1), 38-48. https://doi.org/10.1111/j.1445-6664.2011.00403.x

Venuprasad, R., Impa, S.M., Veeresh-Gowda R.P., Atlin G.N., & Serraj, R. (2011). Rice near-isogenic-lines (NILs) contrasting for grain yield under lowland drought stress. *Field Crops Research*, 123(1), 38-46. https://doi.org/10.1016/j.fcr.2011.04.009.

Villani, P., Demason, D.(1997). Roles of the Af and Tl Genes in Pea Leaf Morphogenesis: Characterization of the Double Mutant (*Afaf tltl*). *American journal of botany*. 84(10), 13-23. https://doi.org/10.2307/2446131.

Wall, D. A., Freisen, G. II., & Bhati, T. K. (1991). Wild mustard interference in traditional and semi-leafless field peas. *Canadian Journal of Plant Science*, 71, 473-480.

Wang, N. (2018). Quality of western Canadian peas 2018. Retrieved from https://www.grainscanada.gc.ca/en/grain-research/export quality/pulses/peas/2018/pdf/2018-pea-report.pdf

Wang, T. F., Gossen, B. D., & Slinkard, A. E. (2006). Lodging increases severity and impact of mycosphaerella blight on field pea. *Canadian Journal of Plant Science*, 86(3), 855-863. https://doi.org/10.4141/P05-094

Wang, X., Singh, D., & Marla, S. (2018). Field-based high-throughput phenotyping of plant height in sorghum using different sensing technologies. *Plant Methods,* 14, 53 https://doi.org/10.1186/s13007-018-0324-5

Wricke, G. (1962) Evaluation Method for Recording Ecological Differences in Field Trials. *Z Pflanzenzücht*, 47, 92-96.

Yahuza, I. (2011). Review of radiation interception and radiation use efficiency in intercropping in relation to the analysis of wheat/faba bean intercropping system. *Journal of Biodiversity and Environmental Sciences*, 1(5), 1-15.

Yang, M. D., Huang, K. S., Kuo, Y. H., Tsai, H. P., & Lin, L. M. (2017). Spatial and spectral hybrid image classification for rice lodging assessment through UAV imagery. *Remote Sensing*, 9(6).583. https://doi.org/10.3390/rs9060583

Yu, J., Li, C., Paterson, A., (2018), High throughput phenotyping of cotton plant height using depth images under field conditions. *Computers and Electronics in Agriculture*, 130, 57-68, https://doi.org//10.1016/j.compag.2016.09.017.

Zhang, C., Taran, B., Warkentin, T., Tullu, A., Bett, K.E., Vandenberg, B., & Somers, D. J. (2006). Selection for Lodging Resistance in Early Generations of Field Pea by Molecular Markers. *Crop Science*, 46(1), 321-329. https://doi.org/10.2135/cropsci2005.0123

Zivanov, D., & Savic, A., Katanski, S., Karagić, Đ., Milošević, B., Milic, D., Djordjevic, V., Vujić, S., Krstic, D., & Ćupina, B. (2018). Intercropping of field pea with annual legumes for increasing grain yield production. *Zemdirbyste-Agriculture*, 105(3), 235-242. https://doi.org/10.13080/z-a.2018.105.030.