10th TENTH CANADIAN PULSE RESEARCH WORKSHOP

PULSES 2016

2016 INTERNATIONAL YEAR OF PULSES

Summaries (or abstracts) the Canadian Pulse Research Workshop

October 25- 28, 2016

Winnipeg, Manitoba

10th Canadian Pulse Research Workshop

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10th Canadian Pulse Research Workshop
2016 Meeting
Winnipeg, Manitoba

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10th Canadian Pulse Research Workshop
2016 Meeting
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10th Canadian Pulse Research Workshop 2016

Winnipeg, Manitoba

Two page summaries are provided only if they were available; otherwise abstracts are included.
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Abstracts: Plenary Speakers

BEAN BREEDING IN THE XXI CENTURY

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The major objectives in most bean breeding programs have changed little over the decades. Yield, quality and pest resistance remain formidable challenges, while new threats like global climate change loom on the horizon. Although the breeding objectives are largely the same, breeders are deploying innovative methods to meet challenges, new and old. Genomic research is at the forefront of this movement, providing new tools and approaches to confront obstacles and exploit future opportunities to enhance nutrition and utilization. The recent genome sequencing of bean genotypes from both gene pools has spurred the development of SNP arrays and allowed for genome-wide association mapping studies, genotype-by-sequencing, candidate gene detection, synteny mapping, and potentially genomic selection for specific phenotypic traits. However, the lack of an effective transformation system for dry beans still limits progress in areas of genetic modification, gene discovery, and verification. Despite the availability of these advanced tools, genetic progress still requires traditional crossing, selection and field testing to identify superior genotypes for varietal release. Integrating these new tools will complement conventional breeding methods, yet maintaining a balance between new and traditional methods will be needed to assure future progress. Bioinformatics, handheld tools that measure photosynthetic processes (e.g. PhotosynQ), and proteomics are just a few examples of cutting-edge technologies that will play key roles in the future as they are integrated into traditional bean breeding programs.

AN ANCIENT SOLUTION FOR 21ST CENTURY GLOBAL CHALLENGES: BUILDING THE CASE FOR INCREASED EMPHASIS OF PULSE CROPS IN HEALTH PROMOTION AND CHRONIC DISEASE INTERCEPTION

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In many parts of the world, pulses are underutilized staple food crops with remarkable yet unappreciated potential to promote health and to reduce the risk for chronic diseases such as obesity, diabetes-type II, cardiovascular disease, and cancer. The discussion of health promotion will focus on the role that pulses should play in closing the dietary fiber gap with presentation of comparative data on the fiber content of common bean, chickpea, lentils and peas. Relative to chronic disease interception, emerging data identifying novel biomedical traits of common bean will be presented as will data on the effects of bean on body fat distribution in a dietary model for obesity. New findings on the effects of whole beans and bean powders on the development of breast cancer in experimental models will also be discussed. Included in this discussion are strategies to evaluate common bean for traits related to obesity and type-2 diabetes, weight loss, and plasma lipid normalization. An opportunity landscape for broadening the impact of pulse crops on human health will be advanced.

Summaries/ Abstracts - Genetics and Breeding

Keynote Speaker – Abstract

ADVANCES IN FIELD PEA BREEDING AND GENETICS

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Pea (Pisum sativum L.) was one of the first domesticated crops, and was the model crop for the foundational genetic studies conducted by Gregor Mendel. Pea is grown in most temperate regions of the world with annual production over the past decade of approximately 10 million tonnes of dry pea and 15 million tonnes of vegetable pea. Most pea breeding activities are conducted in public institutions in Canada, USA, Australia, Europe, India and China, with smaller programs in Africa and South America, and private breeding in companies in Europe, USA and New Zealand. Through these efforts, pea yields have improved by approximately 2% per year over the past 15 years. In addition, substantial progress has been made in improving lodging resistance, disease resistance (fungal, bacterial, and viral), seed visual quality, and modest improvements have been achieved in abiotic (heat, frost, salinity and herbicide) stress resistance. Increased emphasis has recently been placed on enhancing the nutritional quality in pea, and using genomic approaches to facilitate breeding.

GO1

TRACING DOMESTICATION TRAITS IN AN INTERSPECIFIC LENTIL POPULATION

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Cultivated plants differ from their wild progenitors for phenological and morphological traits associated with domestication - germination (dormancy), growth habit (prostrate growth) and seed dispersal (dehiscence). Domestication involved minimizing the effect of these traits, thereby improving productivity. Wild species are increasingly used in breeding as sources of resistance to biotic and abiotic stresses. Breeding research programs aim to minimize the effects of the negative traits by understanding their genetic basis. Lentil recombinant inbred lines (LR-86) derived from a cross between Lupa # 7 (*L. culinaris*) x BGE016880 (*L. orientalis*) were evaluated in five replications in 2016 in the field at the Crop Science Field Lab of the University of Saskatchewan. The following traits were recorded: days to flowering, days to maturity, plant height at maturity, shattering percentage, number of seeds per plant, and seed yield per plant. Percent germination will be recorded after harvest under laboratory conditions. The population was genotyped and mapped using a genotyping-by-sequencing approach. Investigating the genetic and phenotypic variability of this interspecific population will help us identify the molecular mechanisms underlying the quantitative variation for domestication traits in wild and cultivated lentils.

GO2

ESTIMATING THE HERITABILITY OF NON-DARKENING TRAIT AND ITS ASSOCIATION WITH AGRONOMIC TRAITS IN PINTO BEAN

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INTRODUCTION

Size, shape, and colour are major determinants of seed quality in dry beans. The seed coat darkens in commercial varieties of pinto bean with age. Darkened beans are assumed to have low quality and have discounted value in the market (Junk-Knievel et al. 2008). The non-darkening (ND) trait has recently been introduced to the genetic background of pinto bean in order to develop varieties with reduced risk of postharvest seed coat darkening and enhanced seed quality. Introduction of a gene from a donor parent to improve a cultivar for a specific trait can cause undesired changes in other important agronomic traits, as a result of pleiotropy or linkage. The heritability of agronomic traits vary due to genetic variance, environmental effects, and their interaction. Information on heritability can be used to predict the effectiveness of selection for a trait and design the most efficient breeding method for population improvement. The objectives of this study were to: (i) determine variance components and the broad-sense heritabilities of the ND trait and important agronomic traits and (ii) estimate the genetic and phenotypic correlations between the ND trait and important agronomic traits.

MATERIALS AND METHODS

This study was conducted using two different pinto bean populations containing 337 ($F_{4:6}$) lines. Population one was made from a cross between 52, an ND breeding line developed at the University of Guelph, and Stampede, a darkening variety. In total 174 genotypes formed the population one. Population two was derived from a cross between 88, another ND breeding line with a different pedigree, and La Paz, a darkening variety and contained 167 genotypes. Field trials were conducted using a lattice design in three environments; each with two replicates in 2016, at Elora and Woodstock. The genotypes were phenotyped for days to 50% flowering (DTF), days to maturity (DTM), harvestability (HAR), and yield (YLD). Also visual evaluation was used to categorize the genotypes of each population into ND and darkening phenotypes. Combined analysis of variance was done using SAS *Proc Mixed*. Genotypic and phenotypic correlations between traits and heritability of the traits were estimated using *Proc Mixed* in SAS (Holland 2006 and Holland et al. 2003).

RESULTS

In population number one: 66 ND genotypes and 108 darkening genotypes, were identified. In population number two: 49 ND genotypes and 118 darkening genotypes, were found. No significant differences between the ND genotypes and darkening genotypes were found for DTF and DTM in both populations (table 1). No significant differences between two groups of genotypes were found for HAR and YLD in population #1. However, the ND genotypes were significantly different (α=0.05) from darkening genotypes for HARV and YLD in population #2. The ND genotypes had better scores for HARV, but slightly lower yield in population #2. The estimated broad-sense heritability on a plot-mean basis for DTF, DTM, HAR, and YLD were 47%, 66%, 15%, and 24% in population #1 and 84%, 86%, 45%, and 48% in population #2, respectively. The highest positive genetic and phenotypic correlations were detected between DTF and DTM, followed by DTM and YLD, and DTF and YLD, respectively (table 2). The correlation analysis for evaluating possible correlations between the ND trait and other agronomic traits is underway.

Table 1. Analysis of variance DTF, DTM, HARV, and YLD in two populations of pinto bean in 2016 at Elora and Woodstock Ontario.

Source	DTF	DTM	HARV	YLD
Fixed effects				
Population	ns	*	***	*
Seed phenotype(population)	ns	ns	*	*
Seed phenotype(52×Stampede)	ns	ns	ns	ns
Seed Phenotype(88×La Paz)	ns	ns	*	*
Genotype(seed phenotype*population)	****	****	****	****
Random effects				
Environment	ns	ns	ns	ns
Block(environment)	ns	ns	ns	ns
Iblock(environment*block)	****	****	***	****
environment*population	ns	ns	ns	ns
Seed phenotype(environment*population)	ns	ns	ns	ns
Genotype(environment*seed phenotype*population)	****	****	ns	**
Residual	****	****	****	****

Table 2. Genotypic and phenotypic correlations between agronomic traits in two population of pinto bean.

		DTM	HARV	YLD
Seed phenotype	r_G	-	-	-
	r_P	-	-	-
DTF	r_G	83%	0.2%	63%
	r_P	70%	0.9%	28%
DTM	r_G		5%	67%
	r_P		4%	37%
HARV	r_G			30%
	r_P			16%

Reference

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GO3

CHARACTERIZATION OF A PEA RECOMBINANT INBRED POPULATION FOR RESISTANCE TO HEAT AT FLOWERING

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Field pea is a cool-season crop and is sensitive to high temperature especially during its reproductive stage. As both the annual temperature and summer seasonal temperature in Canada had risen by 1.6°C from 1949-2014, it is becoming an increasing interest of studying heat damage to pea in the Canadian natural field environment, which has not been done by any researcher yet.

Field experiments were conducted over two years (2013 and 2014) for the evaluation of flowering and yield component traits in a field pea recombinant inbred line (RIL) population under normal and late seeding (more heat stressful) environments. The 107 RILs were developed from the cross of CDC Centennial (moderately heat tolerant) X CDC Sage (moderately heat sensitive) at the crop development centre, University of Saskatchewan. Our hypothesis was to generate wide genetic variations among traits of our interest and to map the corresponding QTLs via the high-resolution linkage map constructed by the pulse molecular breeding lab at the department of plant sciences, University of Saskatchewan.

The regression result demonstrated that every 1°C rise in the daily maximum temperature during flowering would cause a 69 gm^{-2} yield reduction. Among the yield component traits on the main-stem, pod number was most positively associated with seed yield, followed by thousand seed weight (TSW) and seed number per pod. Days to flowering termination was also positively associated with seed yield under both conditions. A genetic linkage map consisting of 1024 loci with a total coverage of 1702 cM was developed using Golden-Gate and genotyping-by-sequencing methods. Sixteen QTLs were detected in the normal seeding date experiment, 6 for flowering traits, and 10 for yield component traits (Fig 1). Eight QTLs were identified at late seeding, 4 for flowering traits and 4 for yield component traits. The QTLs for the flowering traits, TSW and reproductive node number were different between normal and late seeding, which implies different mechanisms were involved under the contrasting environments.

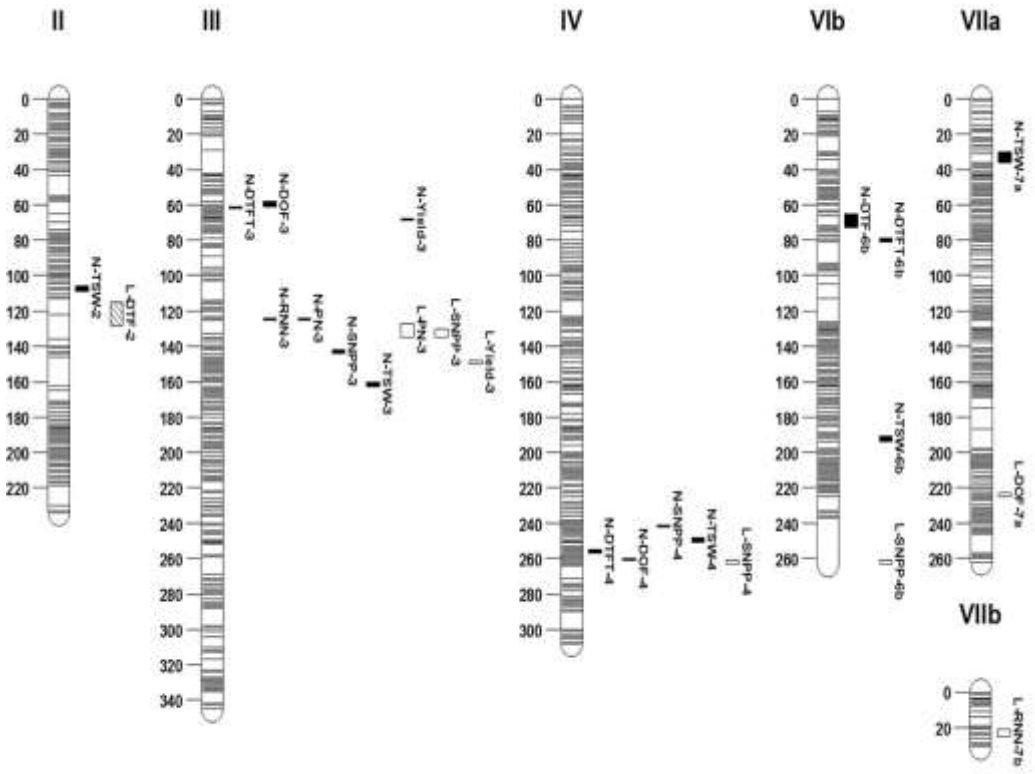

Fig 1. LOD-1 confidence interval of QTLs for measured traits at normal and late seeding date environments in pea recombinant inbred line population-11. Black bars are QTLs for normal seeding date environment. Blank bars are QTLs for late seeding date environment. DTF, days to flowering; DTFT, days to flowering termination; DOF, duration of flowering; RNN, reproductive node number on the main stem per plant; PN, pod number on the main stem per plant; SNPP, seed number per pod; TSW, thousand seed weight (g); Yield, plot yield (g m^{-2}).

GO4
WILD CHICKPEA CROSSES

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Session topic: Genetics and Plant Breeding

Wild relatives of agricultural crops are increasingly utilized as sources of genetic variation. Domestication of chickpeas (*Cicer arietinum* L.) in the Middle East from wild species to our current crop included reduction of genetic diversity through an early domestication bottleneck, followed by highly specialized varieties as part of the green revolution of the 1970s. Investigation of wild plants is based on the interpretation that these are more likely to retain a range of adaptive traits for environmental responses.

In 2014, we crossed CDC Leader and CDC Consul, cultivars developed at the University of Saskatchewan, with 26 accessions of wild chickpea species (20 of *C. reticulatum* and 6 of *C. echinospermum*) collected from Turkey by the Chickpea Innovation Lab (Led by D.R. Cook, UC Davis, USA). Phenotyping and genotyping these interspecific crosses will expand our knowledge of chickpea genetics, including identification of markers to assist breeding programs in retaining specific characteristics. Phenotyping of the wilds and their interspecific progeny is ongoing, with focus on traits of specific interest to western Canadian farmers, including frost tolerance and *Ascochyta* blight resistance.

At the F2 stage, the *C. arietinum* (CDC Leader) x *C. reticulatum* interspecific progeny were screened using KASP markers to select the F2 plants with the cultivar alleles at loci for early flowering and upright growth habit. The selected F2 plants were intercrossed to increase chromosomal recombination. The resulting F1' generation was grown in the greenhouse, where several plants proved to be self-sterile. However, the majority were fertile. Up to ten F2' seeds from each plant were seeded in the summer of 2016. A total of 516 lines produced seed and F3' plants are currently being increased in the greenhouse (Fig. 1). We plan to genotype some of these lines with the ICRISAT 50K Chickpea SNP chip as a nested association mapping (NAM) analysis.

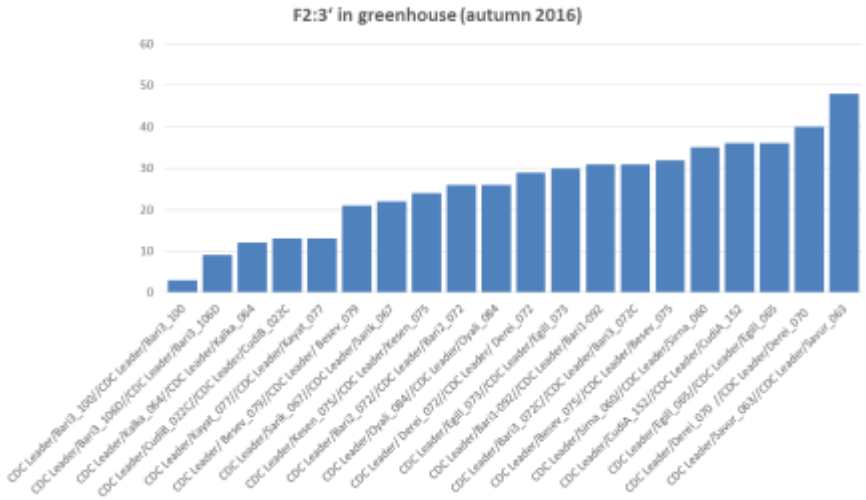

Figure 1. 516 F2:F3' lines currently being increased

Screening for desirable traits included nutrient profiles. The highest levels of iron and zinc were observed in *C. reticulatum* accessions Kesen_075 and Sarik_067 (Fig. 2). Crosses of these accessions with kabuli CDC Leader and desi CDC Consul were done. F4 populations are currently being increased. Phenotyping will be done on the nutrient levels in plant tissues and linkage maps will be developed.

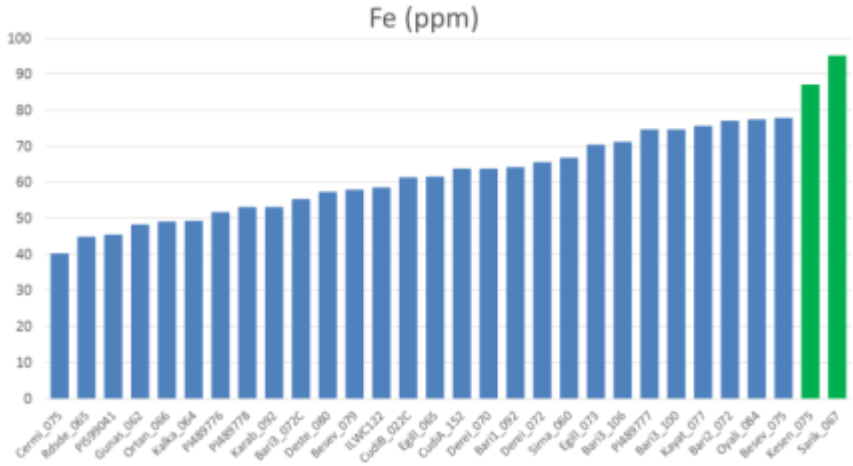

Figure 2. Iron levels in wild chickpea accessions.

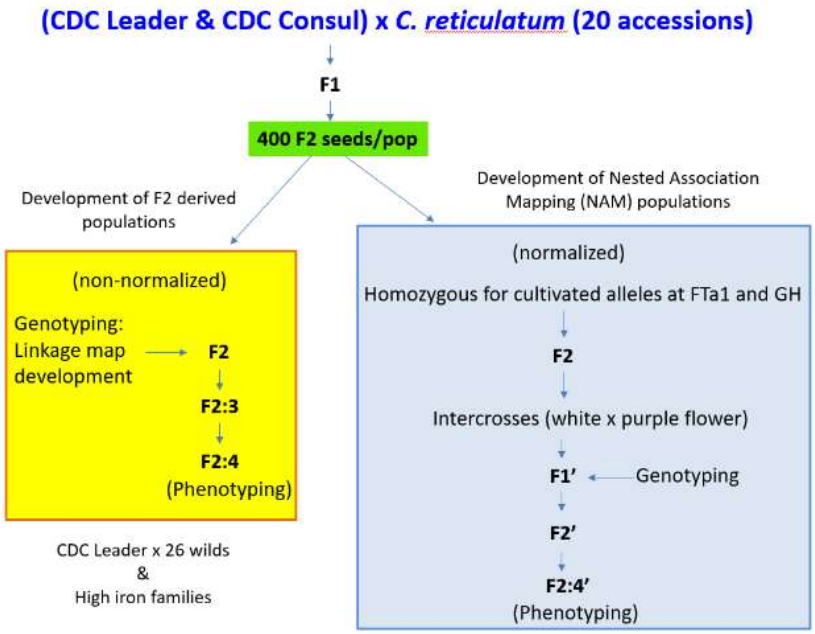

Figure 3. Summary of intentions for interspecific chickpea crosses.

GO5

Phenological study of Lentil (*Lens culinaris* Medik.) in Saskatchewan, Canada

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Introduction

Lentil (*Lens culinaris* Medik.) is one of the oldest food crop grown in the world (Harlan 1992). It is high in dietary fiber, protein, vitamin B and minerals; low in sodium, fat and calories; and free from cholesterol (Bhatty 1988). It is also an excellent source of complex carbohydrates, vegetable protein, and micro-nutrients (Salunkhe et al. 1989; USDA 2016). Thus, lentil is considered part of the solution to combat global food and nutritional insecurity.

Lentil is mainly grown in temperate, Mediterranean and south Asian environments (Fig 1). Unfortunately, lentil breeding program everywhere in the world are based on only a fraction of total available genetic diversity. This is mainly because of the adaptation of germplasm to a specific environment. Adaptation of germplasm is governed by complex interactions of climatic conditions, especially temperature and day length (Summerfield et al. 1985). Un-adapted germplasm flowers at inappropriate times leading to reduced yield.

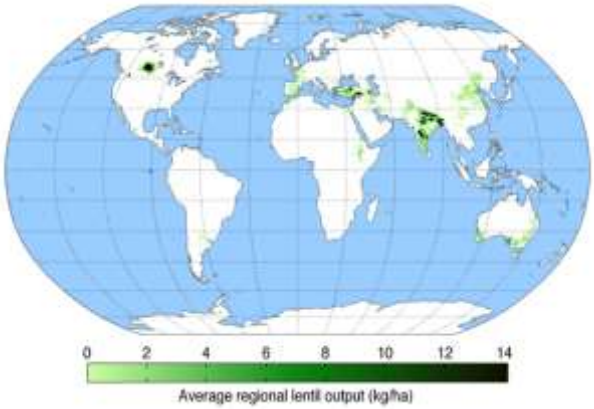

Fig 1: Green color in the map shows the lentil producing areas in the world. The darker green color represents the intensity of the production. https://en.wikipedia.org/wiki/Lentil

Objective

To conduct phenological and agro-morphological characterization of diverse lentil accessions for better understanding of the adaptation response.

Materials and Methods

Three hundred and twenty-four different accessions of lentil, collected from different genebanks and research centers, were tested under field conditions in Sutherland and Rosthern, SK, in the summer of 2016. Field trials were established with 1 sq. meter plots (60 seeds) with three replications at both locations. Data on days to emergence, days to flower, days to swollen pod and days to maturity were recorded and subjected to statistical analyses. Environmental data, including temperature, rainfall and day length, were also collected from the field and used for analyses.

Results and Discussion

There was a range of days to emerge, to flower, to first swollen pod and to maturity across the genotypes seeded at Rosthern (Fig 2). A similar trend was seen in the field at Sutherland and some germplasm even had swollen pods while a few of the later genotypes were still just reaching the flowering stage. This might be because of seeding time difference between the two locations (seeding was a week later in Rosthern).

When we looked at the genotypes based on their origin, we found that the south Asian lines were earliest to flower, the temperate lines were generally later, and the Mediterranean lines ranged from as early as south Asian and as late as temperate lines (Fig 3). The same trend was seen for days to pod swelling and to maturity.

The results support the hypothesis that unadapted germplasm reaches phenological milestones at inappropriate times in SK. Thus, we are going to investigate the interaction between the genotypes and the environment in the other key lentil growing regions to draw final conclusions.

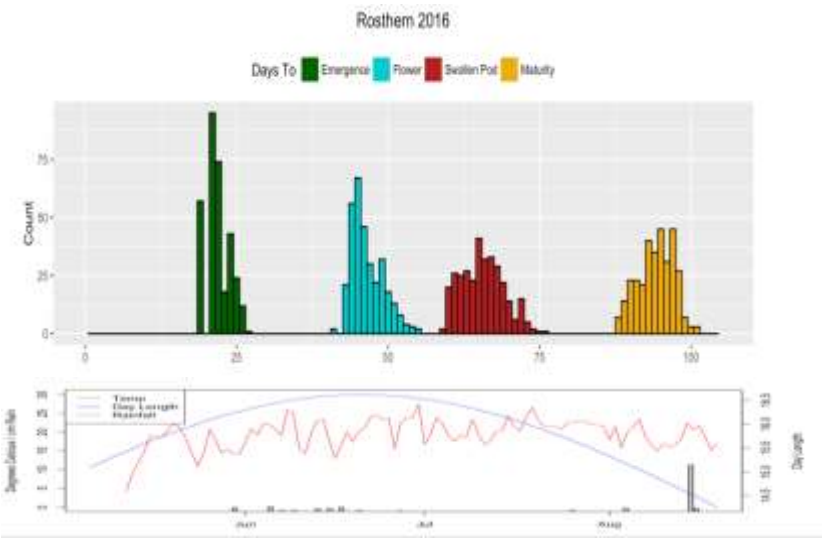

Fig 2: Days to emergence, flowering, swollen pod and maturity (in upper part), daily mean temperature, day-length and rainfall (in lower part) in the field at Rosthern, SK in 2016.

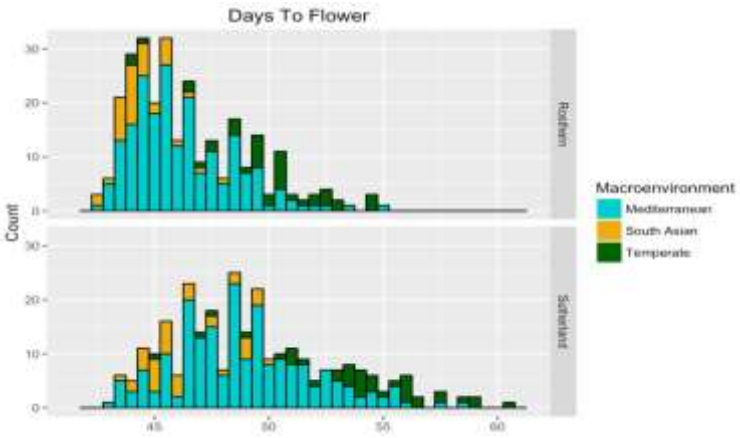

Fig 3: Days to flowering of germplasm based on their originating environment. The upper part is from the field at Rosthern and lower part is from Sutherland.

Acknowledgements

We are thankful to Saskatchewan Pulse Growers, Western Grains Research Foundation, Genome Prairie, Genome Canada and Government of Saskatchewan for providing fund for the research through our 'Application of Genomics to Innovation in the Lentil Economy (AGILE)' project at the University of Saskatchewan.

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GO6

GENOME WIDE ANALYSIS OF NBS-LRR GENES IN CHICKPEA AND THEIR POTENTIAL AS CANDIDATE GENES FOR ASCOCHYTA BLIGHT RESISTANCE

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Plant disease resistance genes are key components of genetic interactions between plant and fungal pathogen and among them a sub-class; Nucleotide binding site and leucine rich repeat (NBS-LRR) is the most common domain involved in governing resistance against a wide range of pathogens. This study tested the hypothesis that NBS-LRR genes are involved in resistance against ascochyta blight in chickpea. Genome wide analysis identified 108 NBS-LRR genes in the chickpea genome that comprises of 0.4 % of total annotated genes. The NBS-LRR genes are not evenly distributed across the chickpea genome and inclined to form clusters. Chromosome 5 has the highest number of the NBS-LRR genes (27% of mapped genes) while chromosome 8 has the lowest number of NBS-LRR genes (4%). A total of 26 NBS-LRR genes were co-localized with the previously reported QTLs for ascochyta blight resistance. Real-time PCR was used to measure relative expression of these 26 genes in three chickpea cultivars (two resistant and one susceptible) at different time points (12, 24, 48 and 72 hours) after inoculation with isolate *AR170*. Differential expression as early as 12 h post inoculation between the moderately resistant cultivars (CDC Luna, CDC Corinne) and the susceptible (ICCV 96029) cultivars was observed. Differential expression observed among the resistant cultivars (CDC Luna and CDC Corinne) at different time points indicating the potential of these cultivars as different sources of resistance. Further efforts to examine the association between NBS-LRR genes with reaction to ascochyta blight infection were done using four recombinant inbred line (RIL) populations derived from crosses between resistant by susceptible and resistant by resistant genotypes under field and greenhouse conditions.

GO7

PERFORMANCE AND NITROGEN FIXATION OF HEIRLOOM DRY BEAN VARIETIES UNDER LOW-INPUT FIELD CONDITIONS

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INTRODUCTION

A distinguishing characteristic of the members of legume family is their ability to form a symbiotic relationship with nitrogen-fixing bacteria and thereby create their own nitrogen source. Soybeans and many forage crops have been reported to fix over 80% of their nitrogen from the atmosphere and therefore do not need chemical N inputs for production (Provorov and Tikhonovich, 2003). Dry beans are considered to be poor nitrogen fixers (Bliss, 1993). Modern production practices see routine use of N fertilizer to ensure reliable yields and standard commercial varieties perform well under these conditions. However, a previous study of 12 standard commercial genotypes at the University of Guelph reported a range for percent nitrogen derived from the atmosphere (%Ndfa) from 11-55 (Farid and Navabi, 2015). Symbiotic nitrogen fixation is downregulated in the presence of a freely available N source and traits associated with nitrogen fixation are difficult to phenotype, therefore modern varieties have not been bred for enhanced nitrogen fixation. Heirloom bean genotypes, passed from generation to generation for centuries, were developed before the advent of modern production and may be a source of alleles which contribute to SNF.

The following study was initiated to examine symbiotic nitrogen fixation (SNF) capacity under low-input field conditions among heirloom and standard dry bean varieties and breeding lines. The objectives were to identify genotypes suited to low-input production and those with breeding potential for enhanced SNF.

MATERIALS and METHODS

A panel of 48 heirloom (H) and standard (S) dry bean genotypes was created. The 28 heirloom genotypes were purchased from Canadian heirloom seed distributers: Heritage Harvest Seed (Carmen, Manitoba), Assiniboine Tipis (Lundar, Manitoba) and Annapolis Seeds (Middleton, Nova Scotia). The genotypes ranged from small to large-seeded, and represented a variety of market classes. The 20 standard genotypes in the panel were taken from the seed stores at the University of Guelph. The genotypes were small or large-seeded, represent modern market classes, and ranged in commercial release date from the 1960s to 2015. The non-nodulating mutant, R99 (Park and Buttery, 1988) was included as the test genotype to determine %Ndfa.

Prior to planting, tests were completed to ensure low N residual in the soil. Seeds were inoculated with *Rhizobium leguminosarum* (Nodulator, BASF Canada) at planting, and the plots were maintained conventionally, except no nitrogen fertilizer was added. An alpha lattice experimental design was used with two reps in each location year; Elora 2014 and 2015, Belwood 2015. Forty-two genotypes were grown each year. A number of agronomic traits were measured. Seed was prepared for ^{15}N isotope analysis (AAFC Lethbridge, Alberta) to determine %Ndfa using the Natural Abundance method (Shearer and Kohl, 1986). Analysis of variance and correlation statistical analyses were performed in SAS 9.3 (SAS Institute). DNA of 39 genotypes was extracted (Macherey-Nagle) and sent to McGill University Genome Québec Innovation Center for SNP genotyping with the Illumina BARCBean6K_3 BeadChip (Hyten *et al*, 2010). To determine the

identity by state relationship of the genotypes, population structure analysis was performed using 20 SNPs per chromosome (220 total) in Structure (Pritchard lab, Stanford). GGT2 was used to visualize the genetic distances between genotypes (Wageningen UR Plant Breeding).

RESULTS and DISCUSSION

Identity by state genetic relatedness analysis found Mesoamerican and Andean heirloom genotypes which grouped with the expected standard genotypes. The Mesoamerican group had higher nitrogen fixation than the Andean group. Although significant differences were seen between genotypes for %Ndfa, the heirloom and standard genotype groups were not significantly different overall. The range of %Ndfa in the heirloom group was larger than that in the standard group (Figure 1). A similar range in %Ndfa was found among genotypes of the Mesoamerican Diversity Panel (Wilker *et al*, unpublished) and the Andean Diversity Panel (Kamfwa *et al*, 2015). The highest heirloom nitrogen fixer was Coco Sophie, a round navy bean-like variety, recorded as early at the mid-1700s in Germany (Heritage Harvest Seed). The highest standard genotype was Hi N Line, a matte black bean, followed by OAC Inferno, a recently developed light red kidney released by the University of Guelph . OAC Inferno has been found in other studies to be a high N fixer (Kamfwa *et al*, 2005). These lines show promise for low-input production and incorporation into a breeding program to develop varieties with enhanced nitrogen-fixing potential.

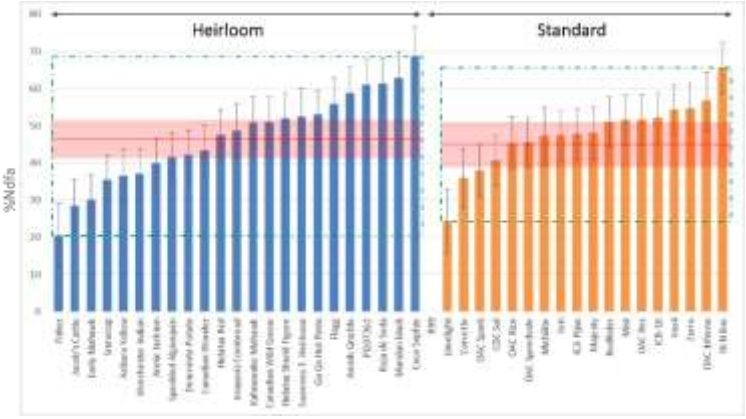

Figure 1. Percent N derived from the Atmosphere. Data presented is a summary of three combined location years. Category means denoted by red line, +/- 95% confidence limits. %Ndfa range outlined in green dotted line.

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GO8

GENES FOR PATHOGENICITY IN XANTHOMONAS AND RESISTANCE IN PHASEOLUS INVOLVED IN COMMON BACTERIAL BLIGHT IN BEAN

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Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. phaseoli and its fuscan variant *X. fuscans* subsp. fuscans, is a damaging disease of dry bean (*Phaseolus vulgris*) throughout the world. The disease can cause yield losses of up to 40% and can reduce seed quality, when the pods become infected. Effective resistance to the pathogen has been introduced into dry bean by crossing with a wild relative, the tepary bean (*Phaseolus acutifolius*; Parker, 1985; Scott and Michaels, 1992). OAC-Rex, which was the first bacterial blight resistant cultivar in Canada (Michaels et al., 2006) derives its resistance from the *P. acutifolius* line PI 440795.

CBB resistance is a quantitative trait, conditioned by loci on several chromosomes. OAC Rex contains a major quantitative trait locus for CBB resistance on chromosome 8, which is associated with the molecular marker SU91. OAC Rex is an important parental line in the bean breeding program at the University of Guelph and has been used as a source of CBB resistance in many crosses leading to high yielding, disease resistant, cultivars released by the program. The genome of OAC Rex was recently sequenced (http://www.beangenomics.ca/) to provide us with the data we need to investigate the molecular bases of a number of traits, including CBB resistance.

Isolates of *X. axonopodis* pv. phaseoli and *X. fuscans* subsp. fuscans show different levels of pathogenicity. The bacteria use a type III secretion system (TTSS) to transport a variety of effector molecules into the host cells (Perry and Pauls, 2011).

The objectives of the current work were to:

1. compare the genome sequence of OAC Rex in the region of the SU91 marker to the genome sequence of the susceptible line G19833, on which the genome sequence for *P. vulgaris* is based; and
2. characterize the genomes of several Xanthomonas isolates with different pathogenicities

The genes in the region containing the marker SU91 on chromosome 8 in OAC Rex were compared to genes in the homologous region in G19833 to identify potential candidate genes for resistance. Polymorphisms were identified in a cluster of R (resistance) genes and in a sterol transport gene. The sterol transport gene, which is homologous to a Niemann Pick cholesterol transporter (Carstea et al., 1997), is complete in G19833 but is split into two genes in OAC-Rex. An alignment of the two genes in OAC-Rex with homology to the Niemann Pick cholesterol transporter in G19833 and Niemann Pick-type genes from *Glycine max* and *Medicago truncatula* showed that the OAC-Rex genes are derived from beginning and end of the gene in G19833, and code for 900 amino acids from the N-terminus and 1100 amino acids from the C-terminus. The intervening sequence in OAC Rex is over 3000 bp in length. Mis-expression of the Niemann-Pick-like protein has been associated with sphingolipid accumulation and reproductive defects in Arabidopsis (Feldman et al 2015). A variety of links between sphingolipid metabolism and plant resistance mechanisms have been noted (Berky et al 2012) and may mediate CBB resistance in OAC Rex.

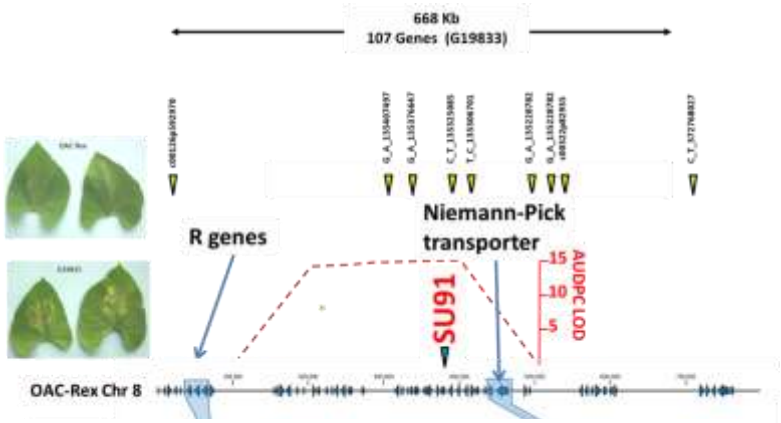

Figure 1. Comparison of OAC Rex sequence in the region of the SU91 marker with G19833 sequence

In addition, a R gene cluster close to SU91 has two additional genes in OAC Rex, compared to the G19833 sequence. The differences in the Nieman Pick and R genes between OAC Rex and susceptible genotypes are being studied in more detail in bean mapping populations and association mapping collections to determine their relationship to resistance.

The diversity of the CBB pathogen was studied by isolating single colonies (lines), from each of four locally collected bacterial isolates, and testing them on a series of bean genotypes to characterize their aggressiveness. The genomes of 7 bacterial isolates were sequenced. Their genome sizes ranged from 5.32-5.36 Mbp and some genomic rearrangements were observed between the isolates. Differences in candidate virulence factors were identified that may be related to differences in aggressiveness between the bacterial lines through interactions with the promoters of target genes in bean resistance genes in the SU91 QTL region.

The information about the molecules that mediate pathogenesis in Xanthomonas and resistance in Phaseolus will facilitate breeding bean cultivars with durable disease resistance.

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GO9

STEPS TOWARD BREEDING FOR IMPROVED NITROGEN FIXATION IN PEA

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Introduction

Most of the biologically fixed N in agri-ecosystems arises from the symbiosis of N-fixing bacteria with legume crops. In 1990s, the amount of nitrogen arising from cultivation of legumes was estimated to be up to 40 million tonnes annually worldwide, providing about 20% of the available nitrogen in agricultural systems globally. Biological nitrogen fixation (BNF) is more desirable than use of N fertilizers due to economic and ecological reasons, especially in pea (*Pisum sativum*. L) which is widely grown in western Canada. The improvement of BNF in agriculture can make a major contribution to both sustainable agriculture. In this study, our objectives are: 1) evaluating collected pea mutants for their growth and BNF capabilities in symbiosis with different *R. leguminosarum* bv. *viciae* strains, and 2) provide useful information for further pea breeding and inoculant development programs.

Methods

Pea germplasm

For field study, two supernodulating lines Frisson P64 *Sym29* and Frisson P88 *Sym28* (which produce large amount of small nodules) along with their progenitor Frisson, one hypernodulating line Rondo-*nod3* (fix+) (which produce many big nodules) along with its progenitor Rondo, and two pea cultivars, CDC Dakota and CDC Meadow, grown in western Canada were tested for their N fixation capabilities under three different field conditions in Saskatchewan. A non-nodulating pea line Frisson P56 (nod-) was used as a non-fixing negative control. For greenhouse study, Rondo-*nod3* (fix+), Frisson P64 *Sym29*, CDC Dakota and CDC Meadow were applied based on results of our field study, and Frisson P56 (nod-) was used as negative control as well.

Field experiments and ^{15}N application

A randomized complete block design (RCBD) with 5 replicates was utilized. Plot size was1 m^2 including the 15cm space between plots (microplot), with each microplot consisting of three rows with 30 cm row spacing. Seeding rate was 60 seeds per m^2. Two weeks after planting when pea plants were in the seedling stage, ^{15}N treatment was applied between the second and third row of each plot, followed by application of 500ml of distilled water to allow the ^{15}N solution to penetrate the top soil layer and spread evenly. The same amount of ^{15}N solution was applied again two weeks after the first application. Overall, the total $^{15}NH_4{}^{15}NO_3$ application in each plot was 0.55 g. Weeds were hand-removed from the experiment during the entire growing season.

Greenhouse assay and experimental design

Selected pea lines' seeds were surface sterilized with 70% ethanol for 5 min and washed with running tap water for 1 min. Surface sterilized seeds were germinated on moist sterile filter paper in the dark at 22 °C for 48 hours. Five gallon pots were used in this study and all pots were fulfilled with top soil that provide by field lab of University of Saskatchewan. Seven tested strains plus one commercial strain (positive control) were used in this study. Top soil was sterilized before potting. At

the day of planting, seeds were sown, two per pot, and each pot was inoculated with one strain *R. leguminoserum* using 1 ml of inoculant containing 10^6cfu ml^{-1}. Negative control only received the same amount of water but no inoculum. After germination, all pots were trimmed into one plant per pot. Proportion of plant N supplied by BNF can be measured using isotopic N ratios based on enriched ^{15}N method.

Results and Discussion

Field study

Considerable variation in yield and BNF was found among tested pea lines. Commercial pea cultivars CDC Dakota and CDC Meadow showed greater biomass and amount of fixed N compared to tested mutants since they were bred for specific adaptation to western Canadian environmental conditions. The mutants Frisson P88 *Sym29* and Rondo-nod3 (fix+) showed greater BNF, higher nodulation and %*Ndfa* among the three tested pea mutants, indicating the potential of these two pea lines as parents in breeding for improved N fixation. Environmental factors significantly influenced the growth and N-fixing ability of the tested pea lines at three sites on the prairies. Environments with moderate soil moisture content, relatively low available N, optimized available P concentration and neutral pH should be the best for differentiating pea lines in terms of N fixation.

Greenhouse study

Cultivar effects on fixed N in pea plants were detected in this study, which may be due to their influence on the composition of *R. leguminosarum* bv. *viciae* in the rhizosphere. Strain effects on nodulation and fixed N in pea plants were also found. Evaluation of cultivar×strain interaction in this study would be beneficial for identifying both superior strains and pea breeding lines with genetic superiority in BNF, which provide useful information for further pea cultivar development and commercial rhizobia inoculant selection in North America.

Further crossing experiment

We used two selected pea lines Frisson P88 *Sym29* and Rondo-nod3 (fix+) as donor parents which crossed with two commercial pea cultivars CDC Meadow and CDC Dakota as recipient parents to yield four F1 crossing lines. These F1 seeds from each crossing line back-crossed with their own recipient parent to yield BC1F1 generation. After 2 times selfed, BC1F3 would be grown under field condition in SPG experimental field in Saskatchewan, in order to select good hybrids with high BNF potential and good yield in near future.

GO10

GENETIC CONTROL OF RESPONSES TO LIGHT QUALITY CHANGE IN AN INTERSPECIFIC RIL POPULATION OF LENTIL

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Abstract

Light, both quantity and quality, is key to plant growth and development. An interspecific RIL population (*Lens culinaris* X *Lens orientalis*), developed from parents contrasting in sensitivity to changes in the red to far-red (R/FR) light ratio, was grown in controlled growth chamber environments that differed in light quality parameters. Varied responses were observed for days to flower, days to mature, plant height and yield. Normal frequency distributions were observed for all traits analyzed except for days to flowering which deviated slightly from a normal distribution under the low R/FR condition. Phenotypic correlations between the traits analyzed were identified. QTLs related to sensitivity to changes in light quality were discovered, which will help with the development of markers that could be used in the breeding program to track this trait. A better understanding of light response will improve our ability to develop lentil varieties that have better adaptation to environments with variable light conditions.

Introduction

Light is necessary for the adaptation of plants to specific environments. Information based on light quality induces a collective response in plants. We used an interspecific RIL population of lentil to characterize variations in growth and flowering responses of the individual RILs to changes in light quality, or more specifically the R/FR ratio, in order to gain an understanding of the genetic basis of light responses in lentil.

Materials and methods

93 sublines derived from a cross between *L. culinaris* Lupa and *L. orientalis* BGE016880 with contrasting responses to light quality change were grown and evaluated in the controlled environment growth chambers. The RILs and the parents were planted in split-plot design with four replicates. Phenotypic data on flowering and growth traits were collected and evaluated. Statistical analysis including Pearson's correlation coefficient among the traits was conducted using SAS. Genomic DNA from the parents and 93 RILs were used to prepare GBS libraries and in-house GBS pipeline was used for data analysis (Wong et al. 2015). Genetic mapping was performed using the JoinMap version 4.0 (Van Ooijen and Vooripps, 2001). Marker order and distances were calculated using Maximum likelihood mapping and Kosambi methods and the linkage map was generated with MapChart (Voorrips, 2002). For QTL identification, the genotypic data was integrated with the phenotypic data of the individual RILs. QTL analysis was performed using WinQTL Cartographer v2.5 (Wang et al., 2012) based on composite interval mapping method (CIM). For each trait, the threshold for LOD score was determined by a 1000 permutation test.

Results and Discussion

The RILs of the interspecific lentil population had a wide range of variation for days to flower, days to mature pod, plant height and yield under the two growth conditions (Fig. 1 - A, B, C and D). Analysis of variance indicated significant differences among the RILs for these traits. Pearson

correlation analysis showed that flowering time, days to mature pod, plant height, and yield were positively and significantly correlated.

A genetic linkage map of the population was developed using 843 SNP markers distributed across 7 linkage groups (Fig. 1E). The map spanned nearly 2000 cM of the lentil genome at an average marker density of 2.25 cM. QTLs for all the traits studied were identified (Fig. 1F): one QTL was common to all four traits and had a high LOD value and explained a high % phenotypic variance. The phenotyping experiment is being repeated.

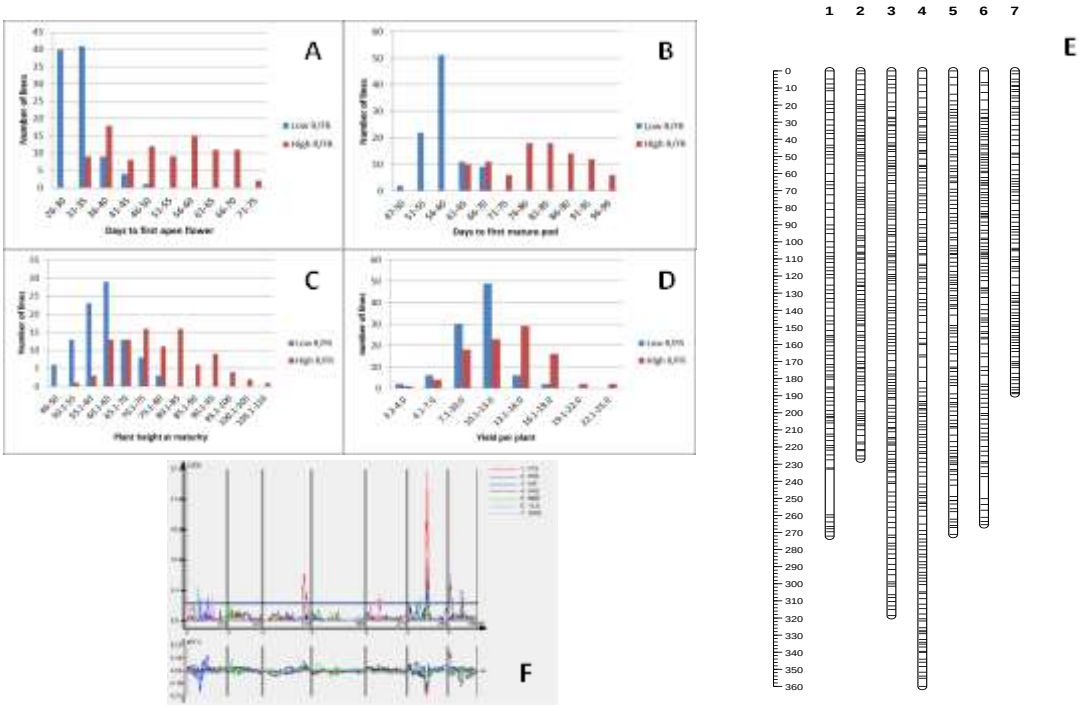

Fig. 1. Frequency distributions for days to flower (A), days to mature pod (B), plant height at maturity (C) and yield per plant (D) of the 93 RIL_S and the parents (Lupa and BGE016880) of the interspecific population of lentil evaluated under low R/FR and high R/FR ratio conditions. Plants were grown at 22°C 16hr light/16°C 8hr night in both treatments. E represents the genetic linkage map of the population and F shows identified QTLs related to the responses to light quality changes on the observed traits.

Acknowledgements

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GO11

GENOME-WIDE STUDY OF CAROTENOGENESIS GENES IN CHICKPEA

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Chickpea is a good source of carotenoid among legumes with diverse germplasm and genome accessibility that make it a good model for carotenogenesis studies. The structure, location and copy numbers of genes involved in carotenoid and isopernoid pathways were retrieved from chickpea genome and compared with *Arabidopsis* and *Medicago truncatula*. Single nucleotide polymorphism (SNPs) study of mentioned genes across five diverse chickpea cultivars showed that majority of SNPs were resulted in synonymous mutation. We also examined the expression of 19 genes in carotenoid pathway and their association with carotenoid concentration at different seed development stages and we observed various expression levels between cultivars. The SNP variations can be associated with the difference in the expression pattern of the candidate genes involved in carotenoid biosynthesis. Genes involved in the primary step of pathway had a significant correlation with various carotenoid components in chickpea.

GO12

GENETIC ANALYSIS OF SEED HARDNESS TRAIT IN A BLACK BEAN RECOMBINANT INBRED LINE (RIL) POPULATION.

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Abstract

Seed hardness trait has a profound impact on cooking time and canning quality in dry beans. This study aims to identify the unknown genetic factors and associated molecular markers to better understand and tag this trait. A recombinant inbred line (RIL) population was derived from a cross between hard and soft seeded black bean parents (H68-4 and BK04-001, respectively). Ninety-two RILs and parents were grown at two locations in southern Manitoba during 2014-15. Seeds from both field and greenhouse grown RIL population were tested for seed hardness traits. The hydration coefficient and stone seed count were estimated by soaking the seeds overnight at room temperature. For mapping of genomic regions contributing to the trait, RIL population was also genotyped using genotype by sequencing (GBS) approach. The QTL mapping revealed that in addition to the major QTL on Chromosome 7 at a genomic location previously reported to affect the trait, another novel QTL with a significant effect was also detected on Chromosome 1. This study also suggests that multiple genetic factors are involved in the control of this complex trait.

Introduction

Seed-hardness in legumes refers to the phenomenon of requiring extended cooking time to allow softening to a desired texture. Seed-hardness trait leads to many detrimental effects such as, increased cost of consumption, loss of nutritional quality, canning quality and uneven germination. Seed-hardness trait is a complex trait, which is affected by both genetic and environmental factors. Genetic factors affecting seed-hardness are not well understood. These factors vary from simply inherited such as *ASPER* (*ASP*) gene that affects seed coat glossiness, to unknown number of major or minor genes. *ASP* gene plays a significant role in seed-hardness. For this reason, matte seed-coat varieties are preferred over shiny ones by industrial processors. The environment impacts seed-hardness by inducing a phenomenon in seeds known as hard-to-cook (HTC) defect. This defect occurs when legume seeds are stored under adverse storage conditions, such as high temperature and high humidity. There are many theories that have attempted to explain the origins of HTC defect, the most well documented among these is pectin-cation-phytate-phytase theory. This theory postulates that the activity of phytase, under adverse conditions leads to degradation of phytic acid causing the release of metal cations. These metal cations, mainly Ca++ migrates to intercellular spaces to bind pectins and thereby rendering them as insoluble pectates. Although environment induced, HTC phenomenon itself is not totally independent of genetic influence, since some varieties are more prone to HTC defect than others. It is possible that similar genetic factors are involved in seed-hardness and hard-to-cook defect. Therefore, to develop superior varieties, it is imperative to understand the underlying genetic factors that render varieties susceptible to seed-hardness and/or of developing HTC defect. The objective of this study was to study the genetic control of seed hardness by using genetic and molecular approaches.

Material and Methods

A black bean bi-parental recombinant inbred line (RIL) population of 95 RILs was developed through single-seed decent by crossing, hard- and soft-seeded parents, H68-4 (P1) and BK004-001 (P2) respectively. H68-4 has seeds with shiny seed coats, and BK004-001 has matte seed coats. The RILs and parents were grown in field (RCBD) at two locations in southern Manitoba and in the greenhouse. The seeds were manually harvested at maturity to avoid mechanical damage caused by machine harvesting. Seeds were airdried to 8-10% moisture before phenotyping. The phenotyping of seed-hardness trait was done by hydration capacity test (AACC method 56-35.01). Hundred seeds from each RIL, were weighed and then soaked in water for 16 h at room temperature. Hydration capacity was calculated from dry and hydrated seed weights. Number of seeds that stayed unhydrated after soaking was counted as stone seeds. RILs were also genotyped using genotype-by-sequencing approach. After filtering, 5056 high-quality SNPs were used for QTL mapping.

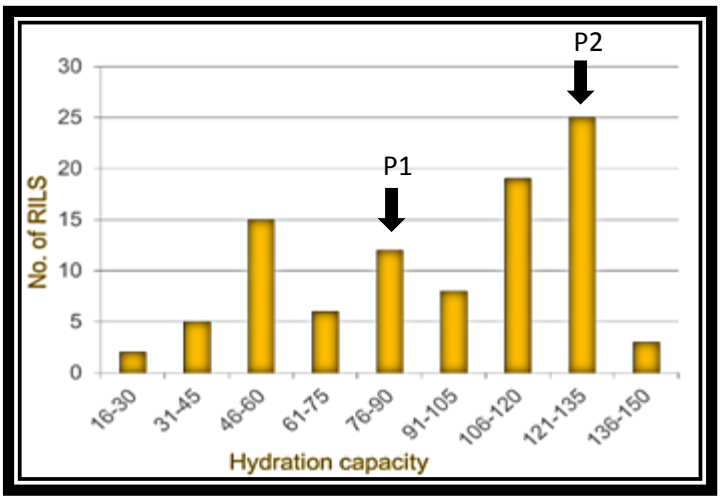

Fig 1. Histogram of hydration capacity values of RILs harvested from Morden field in 2015.

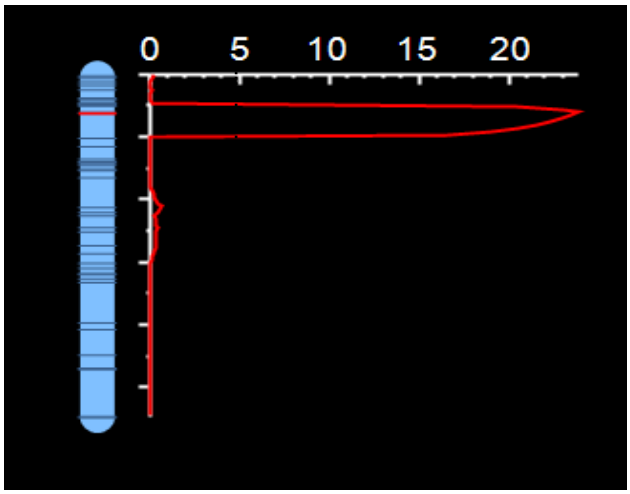

Fig 2. Major QTL for hydration capacity mapped on chromosome 7 explains 45 % of the phenotypic variation.

Results and discussion

Hydration capacity among the RILs was distributed on a wider range than the differences between the parents (Fig 1). Analysis of genotypic and phenotypic data using IciMapping software (Meng et al 2015) mapped three QTLs explaining variation in hydration capacity among the RILs. A major QTL was mapped on chromosome 7 explaining 45 % of the phenotypic variation (Fig 2) and two minor QTLs were mapped on chromosomes 1 and 2 explaining 14.8 and 14.1 % of the phenotypic variation respectively (Fig 3). The location of major

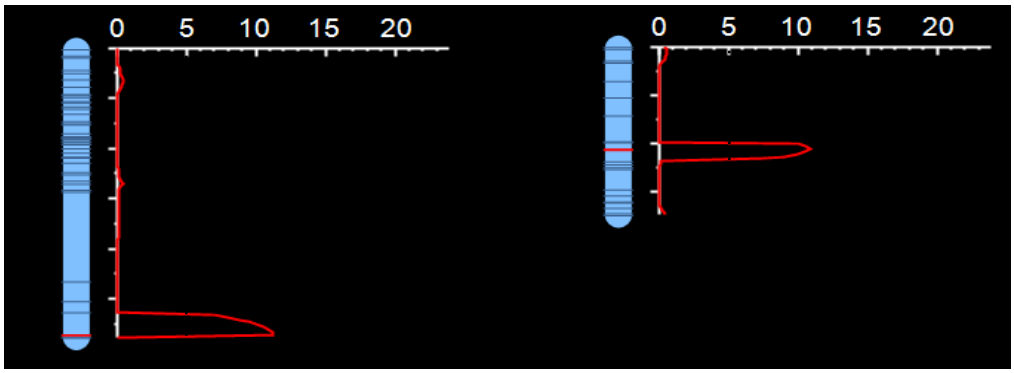

Fig 3. Two minor QTLs on chromosomes 1 (a) and 2 (b) explain 14.8 % and 14.1 % variation in hydration capacity.

QTL on chromosome 7 coincides with *ASP* locus confirming that the two parents segregated for the gene controlling the shiny seed-coat trait (Cichy et al 2015). QTL mapping also confirmed that *ASP* gene plays a significant role in seed-hardness. In addition to the major QTL, we also mapped two minor but significant QTLs on chromosomes 1 and 2. The two minor QTLs also contribute towards seed hardness. Interestingly one of the minor QTLs originates from the soft-seeded parent, explaining the transgressive segregation (Fig 1).

Conclusion

In conclusion, our genetic mapping results show that seed-hardness is an oligogenic trait that is controlled by both major and minor QTLs with significant effects.

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GP13

THE GENETIC AND GENOMIC APPROACHES TOWARDS UNDERSTANDING ASCOCHYTA BLIGHT RESISTANCE IN CHICKPEA

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Chickpea (*Cicer arietinum* L) is an important pulse crop, grown on over 14.9 M ha area with the annual production of 14.3 M tons in 2014 (FAOSTAT 2016). Canada is one of the leading producers and exporters of chickpeas with the production of 169,400 tons of chickpea from 72,000 ha area, which mainly (more than 90 %) comes from Saskatchewan. Chickpea cultivation in the province has been mainly constrained by ascochyta blight disease caused by a necrotrophic fungus *Ascochyta rabiei* (Pass.) Labr. (Taran et al., 2013). The occurrence of ascochta blight disease has been reported in almost all chickpea growing areas worldwide; however, the intensity and severity of the damage have been more in areas with cool growing conditions with the wet environment and a temperature range between 15-25 °C, which are more prevalent in chickpea growing areas of western Canada. The use of resistant cultivar is considered as the most economical method of ascochyta blight management in chickpea. Development of ascochyta blight resistance cultivars has been a major focus of the chickpea breeding program at the Crop Development Centre, University of Saskatchewan however, the quantitative nature of disease resistance, lack of high level of resistance to AB in chickpea primary gene pool, the strong effect of environmental conditions on disease expression (disease pressure) limits this process (Armstrong-Cho et al., 2015; Sharma and Ghosh, 2016).

In recent years significant progress has been made in the development of genetic and genomic resources for chickpea (Deokar et al., 2014). The availability of linkage maps with sequence-based molecular markers (SNPs) and chickpea genome sequence of CDC Frontier has provided a direct comparison of linkage map with a physical map for linking the QTL information on linkage map and physical location of candidate genes on the genome (Varshney et al., 2013; Varshney et al., 2014). Transcriptome profiling using RNA-sequencing (RNA-seq) has provided a platform for simultaneous analysis of genome-wide gene expression and RNA sequencing to discover sequence variants in resistance and susceptible cultivars.We applied QTL analysis, candidate gene mapping, and transcriptome sequencing to study the AB resistance in chickpea. Genomic regions associated with AB resistance were identified using multiple bi-parental recombinant inbred line (RIL) populations on chickpea chromosome 2, 3, 4 and 5. We identified a set of differentially expressed genes in response to *Ascochyta rabiei* infection at three time points (24, 48 and 72 hours post-inoculation) in three chickpea cultivars (ICCV 96029, CDC Luna and CDC Corinne) using RNA-seq. These genes included pathogenesis-related genes, cell wall-mediated pathogen resistance genes, enzymes involved in defense mechanisms and different classes of stress-responsive transcription factors. Integrating information from the expression analysis and candidate genes co-localised in the identified QTLs for ascochyta blight resistance we identified few candidate genes as potentially involved in AB resistance in chickpea.

In conclusion, Integrating transcriptome sequencing information with QTLs mapping provided an effective strategy to identify potential candidate genes associated with ascochta blight resistance in chickpea.

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GP14

GENETIC DIVERSITY AND ASSOCIATION STUDIES OF SYMBIOTIC NITROGEN FIXATION (SNF) IN COMMON BEAN

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Introduction

Common beans are generally considered poor nitrogen fixers compared to other pulse crops, such as soybean and chickpea. Symbiotic nitrogen fixation (SNF) in common bean is being using marker-trait association study in a population of 129 Canadian germplasm entries in a greenhouse and in the field. We used specific nodulator self-adhering peat based inoculant for dry beans (BASF Canada Inc. Saskatoon Canada, previously Becker Underwood Canada) to observe the response of different lines. This study aims to investigate the diversity and ability of diverse bean lines in nitrogen fixing and to perform association mapping analysis using 6K SNP Chip of nitrogen fixation and related traits.

Materials and Methods

A total of 126 lines (supplied by University of Guelph) and three (3) of check lines (mutant lines from OAC Rico) supplied by AAFC, Harrow RDC, ON: R32 – super nodulation and fixing (Buttery and Park, 1990); R69 – super nodulation and non-fixing; R99 – no nodulation (Shirtliffe et al. 1996) were used in this study.

Total nitrogen will be examined to quantify the nitrogen uptake and isotopic N to quantify the proportion of fixed atmospheric N. The ^{15}N isotope method is used to measure biological nitrogen (N_2) fixation in greenhouse and field grown dry bean. We employed the BARCBean6K_3 array developed by Song et al. (2015) to determine the genetic diversity between and within the gene pools for their nodulation and SNF potential. To study SNF at flowering, the 129 genotypes were planted 1 line per growth tube in a 2:1 perlite/vermiculite potting mix. Seeds were coated with inoculum and watered with N free Broughton and Dilworth (B&D) nutrient solution (Broughton and Dilworth, 1971) every other day. The population has been planted in low nitrogen soil at London RDC in summer 2016. Phenotypic data should be available by end of year. The same experiment will be repeated in summer 2017.

Result and Discussion

Population structure result revealed that the population is split into six clusters. Preliminary result based on visual inspection from greenhouse experiment showed that lines with more nodules are mostly grouped in the cluster 4 (ACUG14-C1. ACUG 14-C2, Redhawk, PI414807, Majesty, ACUG13-C1, RedRider and Yeti) and cluster 3 and 5 (Merlot and Windbreaker, Crestwood and AC Cruiser). However, total nitrogen results in roots and shoots from the greenhouse experiment and in seeds from field experiment, are expected to explain the nitrogen uptake and will present clearer idea on genetic diversity toward the pre-breeding program. In conclusion, the discovery will not only contribute to the establishment of genetic diversity in Canadian common bean breeding germplasm, but also will lead to the development of nitrogen-fixing bean varieties through the marker assisted selection (MAS) approach.

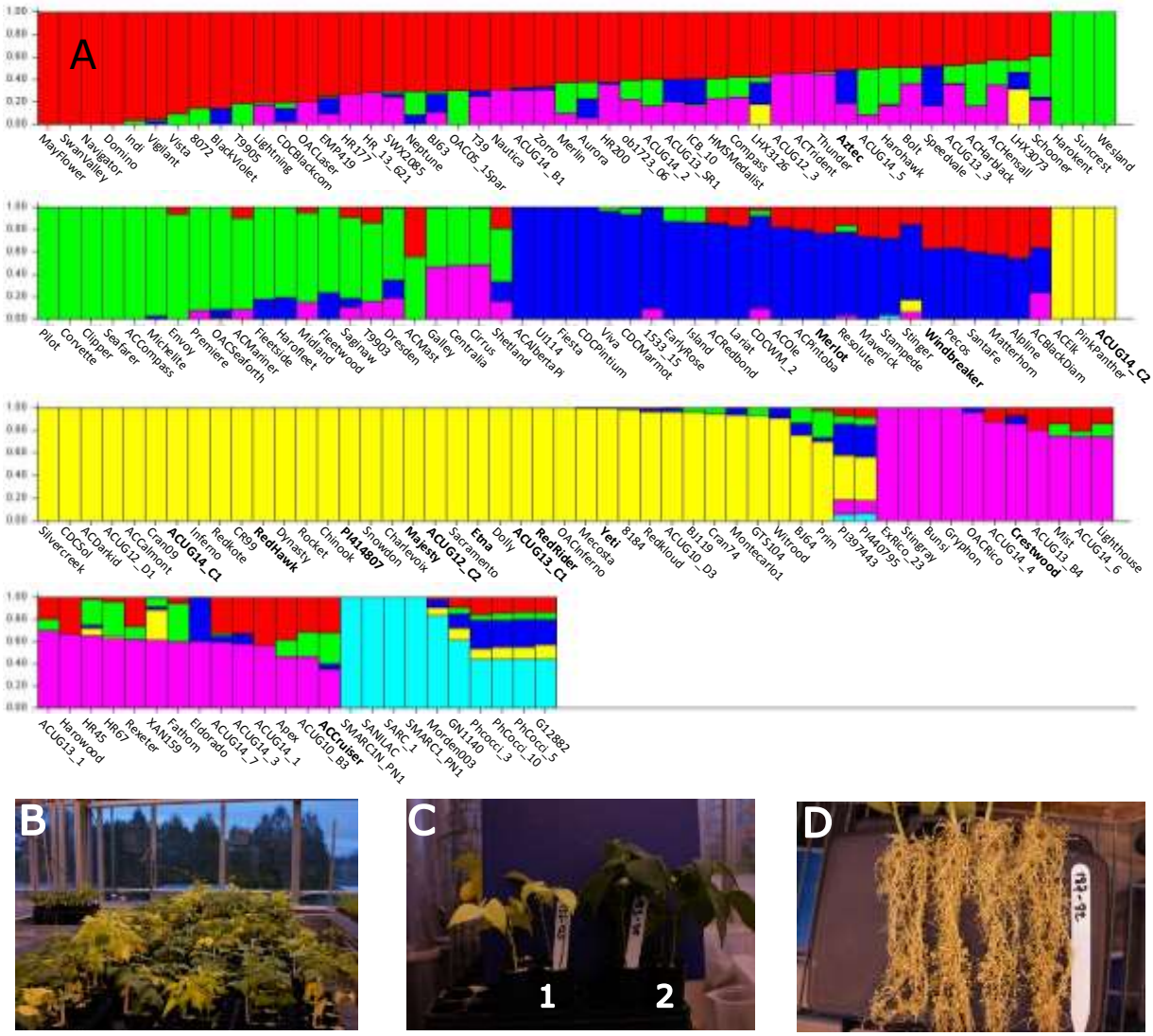

Figure: A) Population Structure of 126 and additional 21 of Canadian common bean genotypes clustered in to 6 clusters, based on 6K SNP chip; B) Greenhouse experiment setting, variation is visible on visual inspection; C) Two contrast lines: 1 (R66) vs. 2 (OAC Rex); D) Nodule appearance on Majesty line.

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GP15

DEVELOPMENT OF SNP MARKERS FOR ASCOCHYTA BLIGHT RESISTANCE FROM AN INTERSPECIFIC *PISUM* POPULATION

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Abstract: Ascochyta blight is an important pea disease which can cause severe grain yield loss. P651 (*Pisum fulvum*) showed the highest level of resistance upon field and greenhouse evaluation of 44 wild pea accessions. To incorporate novel genes for disease resistance, a recombinant inbred line (RIL) population (PR-19) was developed from a cross between Alfetta (*P. sativum*) and P651. These RILs were genotyped using a 1536 single nucleotide polymorphism (SNP) Illumina GoldenGate array and phenotyped at multiple field locations in 2013 and 2014. Overall, nine quantitative trait loci (QTLs) were identified for ascochyta blight resistance, which individually explained 7.5 to 28% of the total phenotypic variation. Among these QTLs, abI-IV-2 and abIII-1 were consistent across locations and/or years. Heterogeneous inbred families (HIFs) populations HIF-173 and HIF-224 were developed to fine map abIII-1 and abI-IV-2, respectively. Under field conditions, HIFs showed a wide range of variation in ascochyta blight scores. HIFs will be genotyped using markers generated from genotyping by sequencing to identify closely linked markers associated with ascochyta blight resistance.

Introduction: Ascochyta blight caused by *Peyronellaea pinodes* (Berk. & A. Bloxam) Aveskamp, Gruyter & Verkley, is the most important disease of pea and causes serious grain yield loss. P651 (*P. fulvum*) was the most promising for resistance breeding as it had relatively low disease score upon field and greenhouse evaluation of 44 wild pea accessions (Jha et al. 2012). The objective of this study was to fine map quantitative trait loci (QTLs) associated with ascochyta blight resistance in interspecific pea population.

Materials and methods: A recombinant inbred line (RIL) population (PR-19) was developed from a cross between Alfetta (*P. sativum*) and P651 (*P. fulvum*). RILs were genotyped using a 1536 single nucleotide polymorphism (SNP) Illumina GoldenGate array (Sindhu et al. (2014) and phenotyped under field conditions in Saskatoon and Rosthern locations in Saskatchewan, as well as under greenhouse conditions. Linkage group was assigned on the basis of the consensus map reported by Sindhu et al. (2014). QTLs were identified using Windows QTL Cartographer 2.5 (Wang et al. 2012). Kompetitive Allele-Specific PCR (KASP) assay was performed to select lines for heterogeneous inbred families (HIFs) development. HIF-173 and HIF-224 were developed to fine map abIII-1 and abI-IV-2, respectively. HIF-173 and HIF-224 were evaluated for ascochyta blight resistance at Saskatoon in 2015 and at Saskatoon and Rosthern in 2016. Fifty one PR-19 RILs were genotyped using genotyping-by-sequencing (GBS) method (Elshire et al. 2011) for fine mapping of abIII-1 and abI-IV-2.

Results: Nine QTLs were identified for ascochyta blight resistance, which individually explained 7.5 to 28% of the total phenotypic variation (Jha et al. 2016). QTL abIII-1 was consistent across locations and years, whereas abI-IV-2 was significant at both locations in 2014. On the basis of KASP assay and disease scores, PR-19-173 and PR-19-224 were selected for development of HIF-173 and HIF-224, respectively. The effect of line was significant ($P < 0.05$) for HIF-173 and HIF-224. Ascochyta blight scores varied from 2 to 9 on a 0-9 scale at physiological maturity stage. The frequency distribution of 126 lines of HIF-173 and 143 lines of HIF-224 indicated a wide range of variation in disease scores. Overall, 10,985 SNPs were identified at a read depth of 10 using GBS

method. After filtering for allele distribution, 7000 SNPs were used for linkage map construction to identify markers within abI-IV-2 and abIII-1 regions. Twenty two and 12 SNP markers were identified in regions next to the closely linked markers within QTLs abI-IV-2 and abIII-1, respectively (Fig. 1).

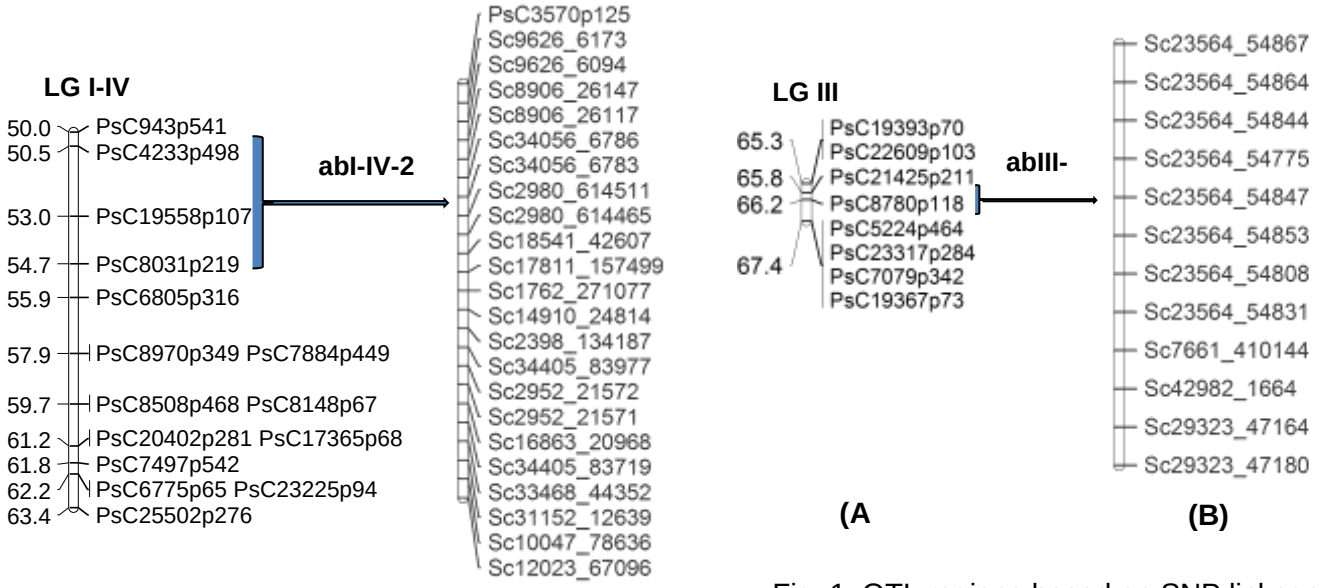

Fig. 1: QTL regions based on SNP linkage map (A), and additional SNPs identified by fine mapping using GBS method (B)

Conclusions and future research: A large number of markers were identified within the QTL regions by fine mapping using GBS and are converted to KASP markers to genotype HIF-173 and HIF-224. Genotypic data will be associated with disease scores to identify SNPs significantly associated with ascochyta blight resistance.

Acknowledgements: The Saskatchewan Ministry of Agriculture, Saskatchewan Pulse Growers, and Western Grains Research Foundation are gratefully acknowledged for financial support. We are thankful to Kamal Bandara, and the staff of pulse crop breeding and pathology for technical assistance.

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G16

DEVELOPING HERBICIDE TOLERANCE IN COMMON BEAN THROUGH GENOME EDITING TECHNOLOGY

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Introduction

Herbicide tolerance is a key trait for crop management. For *Phaseolus vulgaris* or common bean, the herbicide Pursuit (with imazethapyr as active ingredient) is generally utilized pre-emergence or preplant incorporated to control broadleaf weeds. Pursuit is part of the imidazolinone or group 2 herbicides which act as inhibitors of acetolactate synthase (ALS). Other crop species (i.e. canola, wheat, lentil, and soybean [CFIA]) with resistance to this herbicide have been developed and marketed in parts of Canada. These cultivars integrate variants of the ALS enzyme incorporating mutations rendering the enzyme insensitive to the imidazolinone inhibitor acting as the herbicide. The ultimate goal of this project is to develop a common bean variety with resistance to the herbicide Pursuit through the use of genome editing technology.

Materials and Methods

ALS identification: ALS transcripts in the *P. vulgaris* v1.0 genome (and related homologs) were identified using Phytozome v10, aligned using ClustalW, and a neighbour-joining tree was generated to determine relationship of the homologs.

Whole Plant Transformation: Olathe and AAC Burdett bean varieties that were used for this study belong to the Pinto bean market class and have been shown to have higher regeneration rates compared to 25 other common bean varieties previously tested. Cotyledons were dissected out from germinated seeds and placed on media for callus induction and proliferation. Further transformation was performed using a published protocol [1]. Explants were inoculated with *Agrobacterium tumefaciens* EHA105 strain containing pCAMBIA3301 plasmid. Explants were selected with 0.06mg/L glufosinate ammonium.

Results and Discussion

ALS information: We have identified three copies of *ALS* genes in the *P. vulgaris* genome. Basic phylogenetic analysis shows homology between *ALS1* and its soybean counterparts as well as *ALS2* and its soybean homologs (Figure 1). Although *ALS3* groups with *ALS2*, there is some sequence variability that could possibly suggest that *ALS3* may either an allelic variant of *ALS2* or a pseudogene.

Pursuit herbicide tolerance has been previously shown to be induced by mutations in ALS peptides through substitutions in ALS1 (P178S) or ALS2 (W560L) [2, 3]. We determined that in common bean the mutations correspond to G to T transversion and G to A transition in ALS1 and ALS2, respectively.

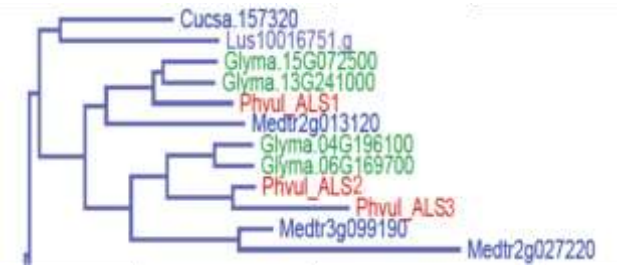

Figure 1: Phylogenetic tree showing relationships of common bean *ALS* genes (red) to their soybean homologs (green). *Medicago truncatula, Cucumis sativa,* and *Linum usitatissimum* are also shown for reference (blue).

Transformation: Using our previously successful regeneration methods, we are in the progress of developing a whole plant transformation system in common bean. Currently in the optimization stages, we will soon be able to determine whether the

Figure 2: Explants on selection media with some showing resistance to glufosinate ammonium.

protocol is successful and if so, determine transformation efficiency of explants that survive the selection stages (Figure 2).

Genome editing: We used common bean genomics information to design guide RNA (gRNA) and donor repair template for CRISPR/Cas9 platform for each of the two *ALS* genes. This will allow us to use genome editing technology to specifically target the area of interest via homology-directed repair mechanism. We have used previously published information on successful editing of the soybean genome and of *ALS* in other species to develop CRISPR/Cas9 platform for our purposes [4-7]. We have transformed pGEM-T Easy vector with gRNA or donor template sequences into *E. coli* DH5α strain. The plasmids have been extracted and are ready for Golden Gate Assembly (pDONR207 plasmid). Assembly of the construct will be done according to a previously published protocol [8]. The construct (Figure 3) will be transformed into the pMDC99 plant expression vector and will be used in further whole plant or hairy root transformation experiments.

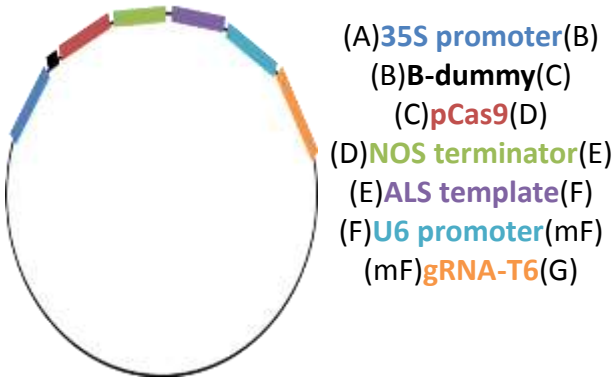

Figure 3: Components of the construct that will be used to produce Cas9 protein, gRNA, and donor template containing mutated site that confers one of the two substitutions.

Summary: We have identified two putative locations on two *ALS* genes (*ALS1* and *ALS2*) which could produce resistant common bean varieties. Initial results of whole plant transformation of common bean explants are promising. Following the completion of CRISPR/Cas9 construct, we will be able to use hairy root transformation system to rapidly show the results in a transient system. The development of whole plant transformation requires a longer period of time, but will be essential to completing the primary research objective. Downstream goals of this project are to apply efficient tissue culture techniques in common bean to developing stable herbicide resistant cultivars for Canadian agriculture in combination with genome editing.

Acknowledgements: Funding for this research comes from Ontario Bean Growers, Manitoba Pulse, Soybean Growers, and Pulse Science Cluster 2 of AAFC.

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GP17

MARKERER ASSISTED BACKCROSS SELECTION OF VIRUS RESISTANCE IN PEA (*PISUM SATIVUM L.*)

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Viruses are a destructive plant pathogen resulting in significant yield loss and reduced grain quality. Pea seed-borne mosaic virus (PSbMV) is an economically important viral disease in pea (*Pisum sativum* L.) and has recently been detected in the Northern Great Plains with significant impact on the industry. PSbMV is aphid-transmitted from plant to plant and can be seed-borne. It causes malformed leaves, discolored or split seed and reduced size and number of seed. Marker-assisted backcross breeding was used to transfer the single recessive resistance allele for PSbMV (*sbm-1*) located on LG VI into locally adapted breeding lines.

Backcross populations were developed using 12 parental breeding lines adapted to North Dakota that were previously developed in the NDSU Pulse Crops Breeding Program. The selected breeding lines will be used as the recurrent parent. Parental breeding lines were hybridized with cultivar 'Lifter' in the fall of 2015 to develop F_1 progeny (fig.1). F_1 plants were selfed or backcrossed to the adapted breeding line as the recurrent parent in the greenhouse in the spring of 2016. Remnant seed from the F_1 plants was hand harvested and advanced in the breeding program as F_2 seed. The F_2 populations were sown in the field at the NCREC at Minot, North Dakota, during the 2016 summer season. The BC_1F_1 population was grown in the field during summer of 2016 near Prosper, North Dakota. Individual BC_1F_1 plants were uniquely identified to allow those testing positive for *sbm-1* to be crossed to the respective recurrent parent to develop BC_2 populations. Successful BC_2 progeny were harvested and planted in the AES greenhouse in the fall of 2016 for generation advancement and PCR testing. The individual BC_1F_1 plants are hand harvested and threshed. Each developed population will be self-pollinated to obtain homozygous recessive individuals conferring resistance to pea seed-borne mosaic virus.

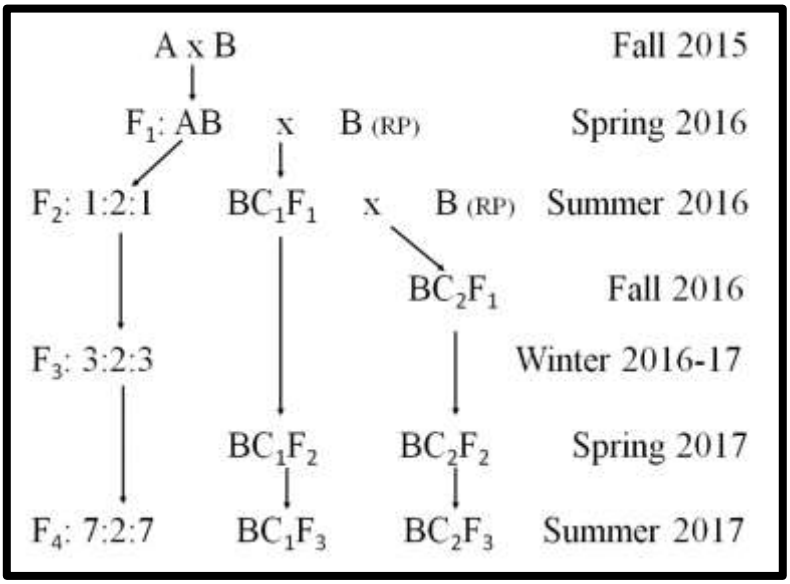

Figure 1: Flow diagram for a marker-assisted backcrossing approach for PSbMV resistance.

The results thus far include DNA analysis on BC_1F_1. The parental lines including 'Lifter' have been identified (fig 2.). Some of BC_1F_1 progeny are included below. Progeny showed a lower band, similar to 'Lifter' (fig. 2), were selected to make crosses to and generate the BC_2 populations. For example, line N16P114 has 6 plants and label 1 to 6, only two of the progeny contain a lower band and will be advanced for development of the BC_2 populations (fig. 3).

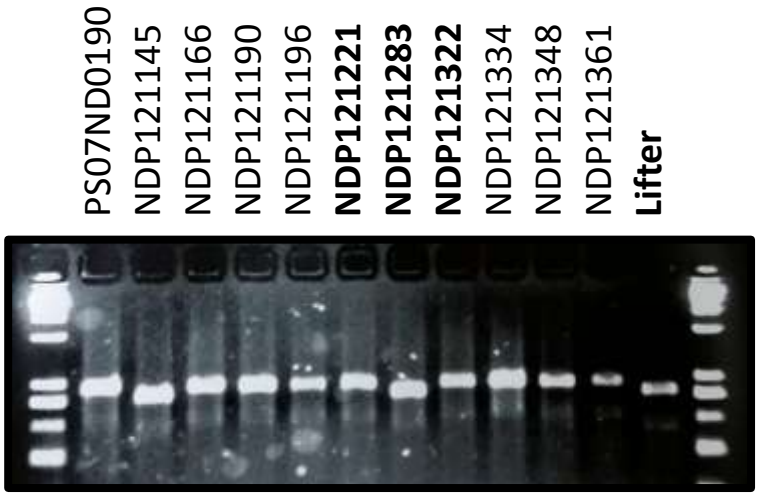

Figure 2: Genotype of 12 parents used in the backcrossing method. The lower band for 'Lifter' identifies the presence of *sbm-1*.

Figure 3: Genotype of selected BC_1 progeny. Bold lanes in 4a are the parents of the BC_1 progeny in 4b.

The study will continue to identify the presence of *sbm-1* in all BC_2F_1 populations. Individuals from BC_1F_2 and BC_2F_2 that test positive to the presences of the gene will be retained and selfed in the greenhouse in the spring of 2017. During the spring, each plant from all populations will be tested again to identify homozygous recessive individuals. The progeny from the BC_1F_2 and BC_2F_2 homozygous recessive individuals will be tested in the field to evaluate for agronomic performance including yield, plant height, and lodging resistance. The single rows will be bulk harvested and yield data recorded for analysis. Data analysis will be performed using ANOVA conducted with SAS. High performing genotypes will be released as germplasm for the scientific community and may be considered for release as new varieties.

GP18

AGRONOMIC TRAIT ASSESSSMENT IN A PHASEOLUS VULGARIS POPULATION OF NAVY BEAN INTROGRESSED WITH P. ACUTIFOLIOUS AND VARIETIES WITHIN THEIR PEDIGREE

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Phaseolus acutifolius (tepary bean) has been introgressed into the background of *P. vulgaris* (common bean) through two separate plant introduction events, contributing the economically important disease resistance to common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. phaseoli and its fuscan variant X. *fuscans* subsp. fuscans, in the Ontario bean breeding program. An agronomic study was conducted to assess more than twenty traits across four sites and included 212 recombinant inbred lines (RILs), developed from a cross between two resistant parents, Rexeter and Apex, three-way crossed to a susceptible parent with no tepary introgression, AC Compass, (AC Compass2*//Rexeter/Apex), as well as thirteen varieties from the ancestral breeding pedigree (including parents). Analysis of the population structure of F1 families screened with a panel of 5830 SNPs, resulted in two primary groupings, those with the susceptible parent (AC Compass) and those without. Plant introductions and resistant parents grouped separately. It is apparent that the discussion of this scope of traits is complex and variable, depending upon the consideration of RILs, varieties, site consistency, the method of assessing traits, etc. Further exploration of the data to determine how traits may have changed over time and whether patterns emerge among those families which group together is underway. This information will be paired with a future genotyping by sequencing assessment to provide a commentary on the potential impact of tepary bean introgression on agronomic traits in a selective breeding program.

GP19

SEED COAT TRANSCRIPTOME ANALYSIS OF TWO PINTO BEAN CULTIVARS THAT DIFFER IN POST-HARVEST SEED COAT DARKENING IDENTIFIES CANDIDATE SLOW DARKENING GENES

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Post-harvest seed coat darkening in pinto bean is an undesirable trait resulting in a loss in the economic value of the crop. During the storage of the bean, seed coat color changes from bright white (left) to pale white or dark (right) as shown in the picture below. The extent of darkening varies between the cultivars and the storage condition.

Figure 1. Photographs showing post-harvest darkening in pinto bean. Pictures were taken for pinto bean cultivars CDC Pintium and 1533-15 (A) immediately after harvest, (B) one year storage at room temperature.

The main objective of this research is to ***identify and characterize gene (s) controlling the post-harvest seed coat darkening in pinto bean.*** Seed coat flavonoid analysis in two pinto bean cultivars: CDC Pintium (regular darkening) and 1533-15 (slow darkening) demonstrated large variation in several flavonoids especially catechin, kaemferol and quercetin.

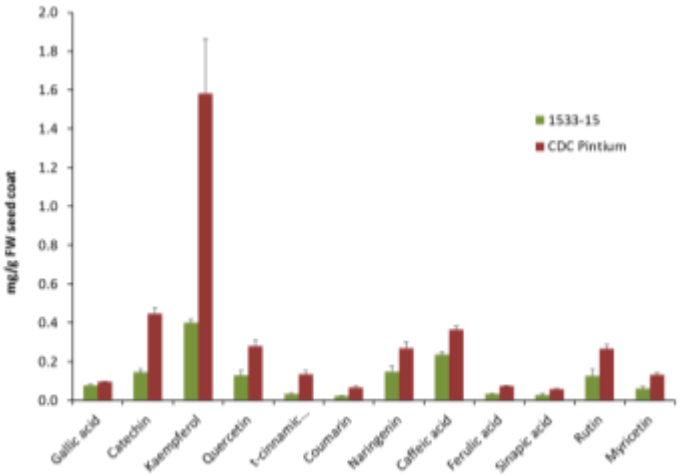

Figure 2. Seed coat flavonoid analysis of CDC Pintium and 1533-15. Seed coat tissue at 150 mg age from both CDC Pintium and 1533-15 were used for flavonoid analysis using HPLC.

In parallel, a comparative gene expression analysis using RNAseq was conducted in these two pinto bean cultivars. The analysis identified several genes such as phenylpropanoid genes, transporters, and those involved in regulation or modification that are differentially expressed between the two cultivars.

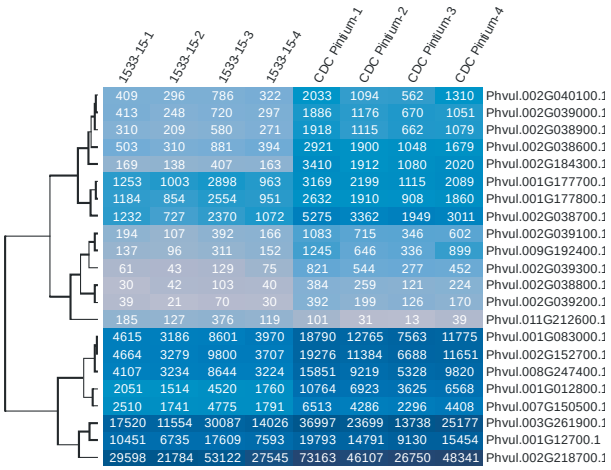

Figure 3. Heatmap of differentially expressed phenylpropanoid genes. Four replicates (R1-R4) of 150 mg stage seed coats from cultivars CDC Pintium and 1533-15 showing expression levels of phenylpropanoid genes. The numbers indicates transcript counts. Locus IDs are as indicated.

Two genes encoding a basic helix-loop-helix (bHLH) transcription factor and a transporter have been selected as potential candidates for playing a role in the seed coat darkening trait in pinto bean.

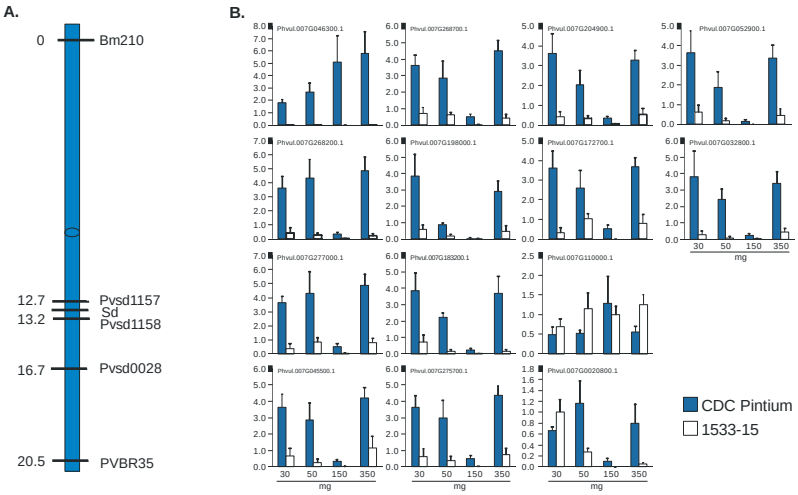

Figure 4. Expression levels of differentially expressed genes located on chromosome 7 during seed coat development in pinto bean. **A.** A schematic diagram showing chromosome 7 in *P. vulgaris* and location of *sd* gene between the two SSR markers (Felicetti et al. 2012, Crop Sci. 52, 1600-1608). **B.** Differential expression of genes located on *P. vulgaris* ch7 in CDC Pintium and 1533-15, measured by quantitative RT-PCR. Data are mean values from two biological and three technical replicates.

The bHLH gene is being characterized to demonstrate its role in proanthocyanidin production and seed coat darkening in pinto bean.

GP20

IDENTIFICATION OF MOLECULAR MARKERS LINKED WITH RESISTANCE TO BACTERIAL WILT IN DRY BEAN

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INTRODUCTION: Bacterial wilt (BW) caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Hedges) Collins & Jones (Cff) can be a serious disease of dry bean (*Phaseolus vulgaris* L.) (Hall 1994). Yield loss due to BW could be 80% in severe conditions. Four Cff color variants have been reported. Use of disease-free certified seeds and disease resistant cultivars are the two best strategies to prevent bacterial wilt (Huang et al., 2009). Identification of resistant germplasm to BW and its use in dry bean improvement are important. However, understanding the genetic control of resistance to BW is difficult as it does not follow classical Mendelian genetics. Identification of resistance to BW in dry bean by molecular markers could be useful for selection. Simple sequence repeats (SSR) are co-dominant DNA markers widely distributed in the genome with multiple copies and are well documented in dry bean (Grisi et al., 2007, Miklas et al., 2007). Identification of such polymerase chain reaction (PCR)-based markers linked to bacterial wilt resistance can be a cost effective solution. Our objective is to identify molecular markers linked with resistance to BW in dry bean.

MATERIALS AND METHODS: Two F_6 recombinant inbred line (RIL) populations derived from Resistant (R) X Susceptible (S) crosses were inoculated and rated for R/S reactions. Seeds were injured near the embryo and were soaked in bacterial suspension for two minutes. Fourteen days after inoculation, 30 seedlings for each RIL were evaluated on a 0-5 scale (Hsieh et al., 2003), where 0 = healthy and 5 = dead seedling. Lines from each population were screened, and categorized into either R or S (R = 0.5 or less, S = >0.5 on 0-5 scale). Genomic DNA was extracted from each RIL (Qiagen Plant DNA kit, Germany). DNA of RILs with a similar phenotypic reaction (R or S) in a population were bulked to produce 25 ng/µl templates and PCR-amplified by SSR primers. After electrophoresis on 2% agarose gels and visualisation (GelDoc XR, Bio-Rad), DNA banding patterns were compared with corresponding R/S reactions. A total of 41 SSR primers have been screened.

RESULTS AND DISCUSSION: Two SSR primers (PVBR-269 and PVRB-35) were found to amplify DNA fragments potentially linked with BW resistance in population 12b (PI 136725 X Kippen), in which primer PVBR-269 and PVRB-35 produced polymorphic bands of 167 and 214 bp, respectively (Fig. 1). However, DNA fragments amplified by these two SSR primers were not linked to BW resistance in population 10b (Early Rose X Kippen). For PVBR-269, resistant RILs produced a band of 167 bp and susceptible RILs produced a band with a larger molecular weight (MW) (Fig. 2). For PVBR-35, resistant lines produced a band of 214 bp and susceptible lines produced a band with a smaller MW (Fig. 2). The DNA fragments amplified in two resistant RILs (line 30 and 40) were similar to the bands in susceptible RILs, which might be due to crossing-over. More SSR primers will be screened to identify new markers linked with BW resistance in dry bean. Genotyping-by-Sequencing (GBS) and validating primers designed from these sequences would also be useful in a future study.

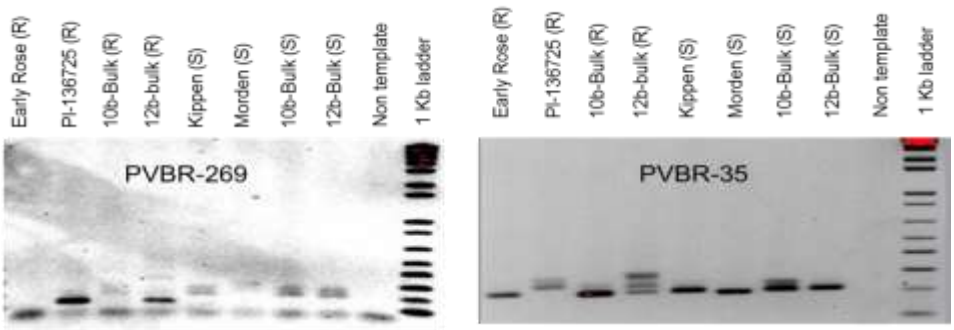

Fig. 1. Bulk segregant analysis for primer PVBR-269 (left) and PVBR-35 (right) for population 12b (PI 136725 X Kippen). Band related to resistance was absent in any of the parental line and RIL showing susceptibility.

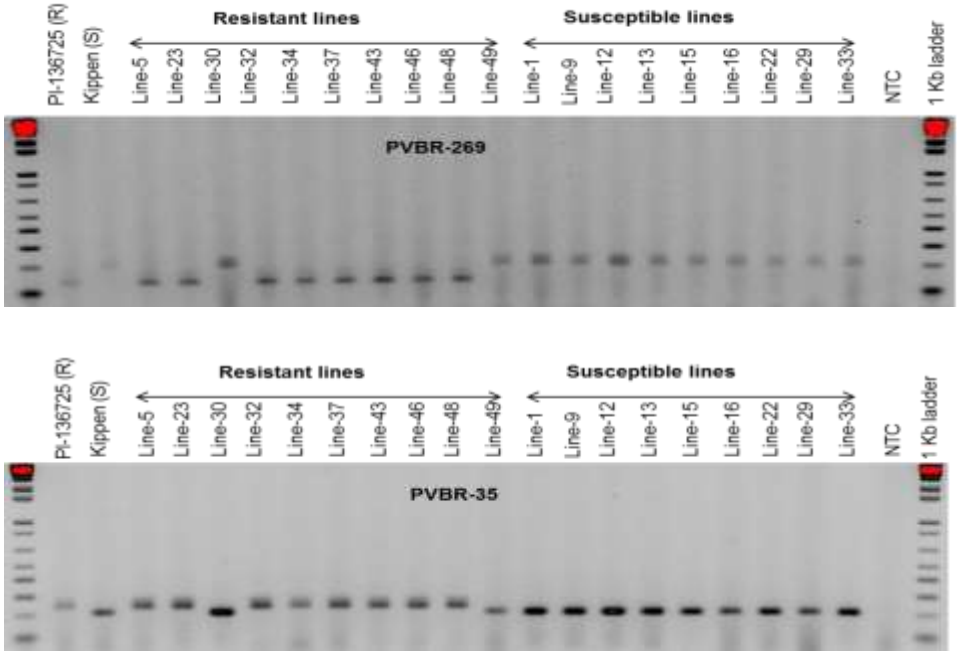

Fig. 2. Marker analysis by primer PVBR-269 (above) and PVBR-35 (bottom) for parental lines and individual RILs exhibiting R/S reaction in population 12b (PI 136725 X Kippen).

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GP21

GENETIC IMPROVEMENT OF PROTEIN QUALITY IN EDIBLE BEANS WITH ADAPTATION TO MANITOBA

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Protein quality in beans is limited by the suboptimal levels of sulphur-containing amino acids, methionine (Met) and cysteine (Cys). The germplasm line SMARC1N-PN1 lacks major seed storage polypeptides. This leads to increased total Cys (up to 70%) and Met content (about 10%) and decreased levels of *S*-methylcysteine as compared with the corresponding wild-type line. A cross was made between SMARC1N-PN1 (S) and the navy cultivar Morden003 (M) to generate an $F_{2:8}$ population of 185 recombinant inbred lines (RIL). Protein profiles classified them into four groups according to genetic inheritance at the phaseolin and lectin loci. Lines were tested under field conditions and their amino acid concentrations were evaluated. Some SS lines were characterized by a 50% increase in Cys concentration as compared with Morden003. This was apparent at the London site, under non-sulphate fertilized conditions, but less so at the Morden site, where sulphate fertilizer is routinely applied, with an increase of 10-20%. The significance of these results will be discussed.

GP22

HIGH PROTEIN PEAS WITH NOVEL STARCH MORPHOLOGY, COMPOSITION AND THERMAL PROPERTIES

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Field pea (*Pisum sativum* L.) is cultivated in many regions of the world and its production ranks the fifth in the world for food legumes after soybean, peanut, drybean, and chickpea. Canada is the largest field pea producer and exporter in the world, with an annual acreage of approximately four million acres and an annual production of almost three million tons. Field pea is mainly used as a protein source, as it has a relatively rich and unique protein profile, different from other natural protein sources. Field peas normally contain approx. 23% protein. Dr. Bing in AAFC-Lacombe identified and utilized novel pea germplasm which contain approx. 30% protein in his breeding program. As the results, Dr. Bing has developed several advanced pea lines with approx. 30% protein, semi-leafless, earlier maturity and larger seeds. In this study, we compared starch morphologies, compositions and thermal properties between high protein peas and other market types of field peas (yellow, green, maple and marrowfat peas).

We used NIR to analyze the protein and starch contents in pea seeds. The mean protein contents were 24.28%, 25.56%, 25.26% and 26.21% for pea types of yellow, green, maple, and marrowfat, respectively. The mean protein content of high protein peas was 32.73%, significantly higher than those of other pea types. By contrast, the total starch contents were 47.52%, 47.02%, 45.96% and 45.87% for pea types of yellow, green, maple and marrowfat, respectively. However, the total starch content of high protein peas was 30.31%, significantly lower than those of other pea types. These results indicated that there was a negative correlation between protein and starch contents in field peas.

We found that there were significant differences in amylose contents between high protein peas and other pea types. The mean amylose contents in starch granules of yellow, green, maple, and marrowfat peas were about 50.46%, 54.37%, 53.46% and 50.47%, respectively. However, the mean amylose content in starch granules of high protein peas was approximately 82.58%. Our results indicated that the amylose contents of high protein peas were significantly higher than those of other pea types, but there was no significant difference in amylose contents among yellow, green, maple, and marrowfat peas.

The degree of polymerization (DP) of amylopectin in field peas was analyzed and the majority of amylopectin chain lengths in starch granules of field peas ranged from 11 to 30 glucose units. The percentages of DP 11-30 were 77.76%, 78.73%, 77.5%, 77.55% and 72.7% for yellow, green, maple, marrowfat and high protein types, respectively. In addition, the percentages of DP 11-20 in high protein peas were significantly lower than that in other pea types.

We observed the morphology of starch granules in field peas under SEM and found that the granular sizes and shapes were significantly different between high protein peas and other pea

types. In general, field peas had starch granules with oval or kidney-like shapes, but the starch granules in high protein peas showed the compound structure with irregular and polygonal shapes. There were some fissures on the granule surfaces of most pea starches from yellow, green, maple and marrowfat peas, whereas the cracks were much deep and obvious on the granule surfaces of high protein peas. The large compound granules of high protein peas were easily subdivided and fragmented into several small irregular and polygonal granules along the cracks.

The granular diameters of pea starch were determined and the majority of starch granules in high protein peas ranged from 3 to 10 μm in diameter, whereas the sizes of starch granules in other pea types were mainly distributed at 5-20 μm. From 5 to 10 μm, there were approximately 36.44%, 34%, 37.09%, 34.57% and 53.37% of total starch granules for pea types of yellow, green, maple, marrowfat and high protein peas, respectively, in which the granule percentage in high protein peas was significantly higher than that in other pea types. However, the granule percentage of 10-20 μm diameters in high protein peas was significantly lower than that in other pea types. Overall, the granular size of high protein peas was significantly smaller than those of other pea types, but there were no significant differences in granular shapes and sizes among yellow, green, maple and marrowfat peas.

The thermal property of pea starch was measured by DSC. The peak temperature (Tp) and enthalpy change (ΔH) of starch gelatinization in yellow, green, maple and marrowfat pea peas were distributed in 66.76 - 67.41°C, and 4.91 - 5.3 J g^{-1}, which indicated that there was no significant difference in starch thermal properties among these pea types. However, the Tp and ΔH values of high protein peas were 79.8°C and 1.99 J g^{-1}, respectively. Our data analysis showed that the To, Tp, and Tc of high protein peas were significantly higher than those of other pea types, whereas the ΔH of high protein peas was significantly lower than those of other pea types. In addition, the temperature range of starch gelatinization in high protein peas was significantly wider than those of other pea types.

GP24

HOW CROP-WILD INTROGRESSIONS MAY AFFECT SEVERAL IMPORTANT AGRONOMIC TRAITS IN LENTIL.

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Like other ancient self-pollinating crops, the gene pool of lentil (*Lens culinaris* Medik. subsp. c*ulinaris*) (Lcu) has become very narrow following domestication and adaptation to specific regions of the world. This has limited the genetic variability available to lentil breeders. To increase genetic variation, wild relatives could be important genetic resources, and crop-wild introgression breeding is being used in some crops for this purpose.
To assess the potential of introgression breeding in lentil, it is essential to investigate the effect on phenotypes from recombination between the genomes. *L. ervoides* (Ler) is one of the wild relatives with great potential in lentil improvement. It has been reported to carry multiple lentil disease resistances (Tullu et al. 2006; Fiala et al. 2009; Vail and Vandenberg, 2010), and the resistance can be successfully introgressed via inter-specific Lcu x Ler crossing (Vail S., 2010; Vail and Vandenberg, 2011).

In this study, we aimed to investigate the phenotypic variability of several quantitative traits of interests that were segregating among individuals of a Lcu x Ler RIL population, LR-26. These traits included days to emerge, vegetative period, reproductive period and plant height as well as a thousand seeds weight. Analysis of variation and estimates of heritability of these traits were based on a three-year multi-environment field trial. Through this study, we wish to understand the phenotypic divergence of crop-wild introgression of this population and collect the useful phenotypic data to be combined with genotyping result of LR-26 from genotyping by sequencing (GBS). This will allow us to identify regions of Ler that have been introgressed and tag genetic regions associated with control of these traits of interest.

GP25

TECHNOLOGY PLATFORM FOR COMPREHENSIVE NUTRITIONAL PROFILING OF SEEDS

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ABSTRACT: Breeding pulse crops with enhanced levels of nutritional components like protein, starch, fiber, carotenoids, iron, zinc, selenium and low levels of anti-nutritionals like phytic acids, is important for marketing premium quality grain, processed foods and feed. Current methods for quantifying these components are relatively low throughput and expensive. We are developing a rapid and cost effective screening technique for these components, applicable directly on field pea seed flour. Synchrotron based X-ray fluorescence and mid-IR absorption spectroscopy will be used to quantify mineral nutrients, and organic nutrient components respectively, in collaboration with the Canadian Light source, Saskatoon, Canada. Spectroscopic results from a wide range of genotypes will be analyzed and interpreted, based on data collected from different pea seed fractions and standard compounds. A standard protocol for a rapid, two step assay of the nutritional components will be developed and validated against industry standard wet bench methods. We have analyzed the nutritional components of several pea seed samples and standards and established preliminary data processing methods towards accomplishing this goal. When fully developed, and adapted, we expect this spectroscopic method to enable routine nutritional phenotyping of pea seed samples and fractions, and other pulses and cereals also.

INTRODUCTION: Superior nutritional quality is important for marketing premium quality grain, processed foods and feed. Nutritional components like protein, starch, fiber, carotenoids, iron, zinc, selenium and anti-nutritionals like phytic acids have been identified by nutritionists and breeders as key factors in defining grain nutritional quality in pulse crops like pea, and also in cereals. Therefore, breeding quality grain with optimal concentrations of high value nutritional components and low levels of the anti-nutritionals can greatly enhance the value of the crop to the industry.

Currently, biochemical/nutritional traits of crops are neither routinely nor extensively used in crop variety development, because multiple, time consuming, and expensive wet bench methods are required for accurately measuring them. This project proposes to develop a rapid and low cost biochemical nutritional profiling (chemotyping) method to accurately measure nutritional quality of the grain using well established spectroscopic techniques (Sarret et al., 2013; Miller & Dumas, 2006; Iwai et al., 2012**)**. These seed biochemical/nutritional profiles would be generated in the month after harvest each year, providing timely data for selecting breeding lines to advance. The mid-infrared (Mid-IR) and X-ray fluorescence (X-ray) spectroscopy methods developed in this project will enable breeders and processors to routinely screen seeds for their total nutritional profile in a cost effective manner. This will set the stage for high throughput evaluation of large breeding populations and germplasm collections, to select genotypes that possess superior nutritional qualities. Once this technology is validated in an important crop like pea, it can be relatively easily applied to other grain crops also.

Results: Preliminary data collected using mid-infrared and X-ray spectroscopy techniques at the Canadian Light Source (CLS) in a previous ADF research grant using pea seed samples revealed that we can accurately identify and quantify comprehensive nutritional profiles of pulses and cereals rapidly and simultaneously. 131 pea seed samples from field experiments in years 2010 to 2013 were sourced for analysis and calibration of the X-ray and Mid-IR spectroscopy based assays of the following components of pea seeds (Table 1). 12 seeds per sample were first pulverized to coarse powder using 50 ml grinding tubes and 2 large (16 mm) ceramic grinding balls and further ground to fine powder in 15 ml grinding tubes with 30 small (5 mm) ceramic grinding balls in a Genogrinder 2010 milling machine (SPEX Sample Prep LLC, Metuchen, NJ, USA). 120 mg seed powder per sample were pelleted into discs for XRF analysis and 1 mg of seed powder mixed with 99 mg of potassium bromide diluent were used for generating compressed pellets for FTIR spectroscopic analysis respectively. XRF Spectra were collected at IDEAS beamline at Canadian Light Source (CLS), Saskatoon and mid-IR spectroscopy was conducted on a IFS 66 spectrometer (Bruker, Ettlingen, Germany) at the CLS.

Table 1: Range known nutrient components in test seeds

	Available Range		assay
Component	Low	High	
Se (ppm)	0.08	6.94	AAS
Zn (ppm)	14.39	53.78	AAS
Fe (ppm)	25.71	93.68	AAS
Phytate (µg/gm)	8	1131	Wades
Total carotenoids (ug/g)	5.37	27.79	HPLC
CP (%)	18.4	31.4	NIR
Ash (%)	1.62	6.48	NIR
EE (%)	0.43	2.7	NIR
ADF (%)	3.1	8.6	NIR
NDF (%)	7.4	20.6	NIR
Starch (%)	7.2	56.85	NIR

The mid-IR absorption spectra of CDC Bronco seeds were recorded between the wave numbers 4000 – 600 cm^{-1} and contain all the major spectral features found in biological samples. The -OH stretching vibration predominantly representing the water content and the -OH groups of carbohydrates (3500 – 3300 cm^{-1}), the region representing vibrations of CH_3, $-CH_2$ bonds of lipids (-3000 – 2800 cm^{-1}), the two prominent sharp peaks representing amide I and amide II vibrations of proteins (1700 – 1600 cm^{-1} and 1600 - 1500 cm^{-1}), a 1510 cm^{-1} shoulder on the amide II peak corresponding to the lignin aromatic ring vibration and the spectral peaks representing the C-O-C, C-C and P-O bonds that are likely to be found in polysaccharides and phosphates of plant samples (in the region 900 to 1250 cm^{-1}) were identified. We have also identified four very strong peaks at 980, 966, 931 and 841 cm^{-1} that represent the P-O-C asymmetric stretch and peaks in the region from1180 to 1126 cm^{-1} that may be associated with phytic acid content of seeds. The averaged XRF spectra of pea seeds indicated that Zn, Ca, Fe, K and Mn content could be directly detected in pellets of seed powders with ease.

Conclusions: The results generated through this study give us confidence that reliable data on organic nutritional and anti-nutritional components of seeds such as proteins, starch, and phytic acid can be effectively combined with valuable complementary information on mineral composition of pea and other crop seeds using the resources available in a synchrotron facility such as the Canadian Light Source. These studies also provide a gateway into the process of precisely localizing mineral nutrients to specific tissue and cellular compartments and correlating the localization with their chemical status and provide valuable insights into the nutritional and processing aspects of crop utilization. Once validated, breeders, biotechnologists and processors will be able to utilize this information to devise knowledge based strategies to improve the nutritional composition of the seeds and seed based products.

In addition, the mid-IR and X-ray spectroscopic information generated in this project can be used to develop a medium to high throughput method to screen seeds of pea and other pulse crops for total proteins, lipids, and different carbohydrates such as starches and pectin (soluble fiber) as well as their mineral components. These preliminary experiments clearly demonstrate that a judicious combination of mid-IR and X-ray absorption spectral measurements can eventually lower the cost, and accelerate the speed of phenotyping seed biochemical traits such as phytate, minerals and other nutritional and anti-nutritional seed components in a breeding program.

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GP26

QTL AND CANDIDATE GENES ASSOCIATED WITH COMMON BACTERIAL BLIGHT RESISTANCE IN THE COMMON BEAN

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Introduction Common bacterial blight (CBB), incited by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (*Xap),* causes severe losses in seed yield and quality in common bean (*Phaseolus vulgaris* L.). The most effective and economical way to control this disease is to use resistant cultivars (Navabi et al., 2011). To identify genomic regions contributing to CBB resistance, a resistant F4:5 recombinant inbred line population of the reciprocal crosses between OAC Rex and HR45 was evaluated under artificial field inoculation in disease nurseries in 2009 and 2010 (Durham et al., 2013) (Fig.1). To develop co-dominant and tightly linked markers to the gene controlling resistance to CBB, a genome-wide SNP marker screen was applied to this population (OAC Rex/HR45). In general, the purpose of this work is to discover and validate regions associated with CBB resistance.

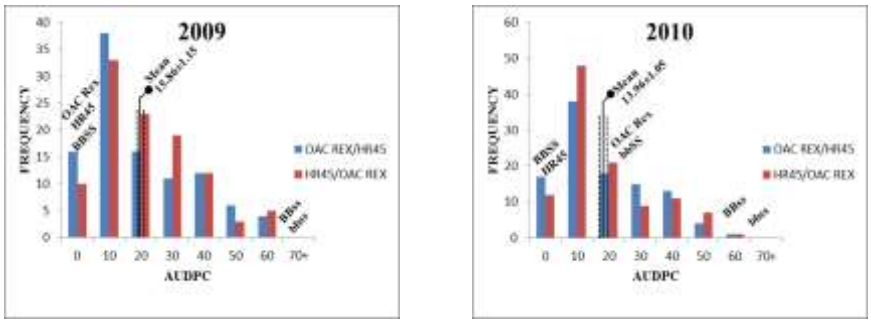

Figure 1. The area under the disease progress curve (AUDPC) evaluated for 218 recombinant inbred lines (RILs) of the reciprocal crosses of OAC Rex/HR45 in 2009 and 2010. The check genotypes for presence and absence of BC420 and SU91 markers are shown above their respective bars. BBSS check for the marker BC420 and SU91, bbSS check for SU91 only, BBss check for the marker BC420 only, bbss no marker (Durham et al., 2013)

Materials and methods The single nucleotide polymorphism (SNP) genotyping of the population of 218 RILs of the reciprocal crosses of OAC Rex/HR45 was performed at the McGill University and Génome Québec Innovation Centre using the Illumina Infinitum BeadChips BARCBEAN6K_1 and BARCBEAN6K_2 with 5,232 and 5,514 SNPs, respectively. JoinMap software was used to construct the linkage map. The Map was calculated using a Maximum Likelihood mapping method with minimum logarithm of odds (LOD) = 5. A physical map was also used as reference to create the linkage groups. Windows QTL cartographer v2.5 was used to conduct the QTL analysis by applying composite interval mapping analysis.

Results

Eight hundred and eighteen SNPs from an Illumina Infinium array comprising a total of 5,232 SNPs were mapped. Composite Interval Mapping identified several QTL accounting for 45% of the phenotypic variation for CBB severity (Table 1,2).

Table 1. Summary of the markers used for the construction of the OAC Rex and HR45 linkage map.

Marker types	Number of screened markers	Number of polymeric markers	Number of mapped markers
SNPs	5398	883	529
SCARs	13	13	4
STS	1	1	1
Total	5412	897	534

Table 2. Linkage map information

Linkage group	Map length (cM)	Number. of markers	Marker Density (CM/Marker)
PV1	16.58	15	1.10
PV2	39.33	21	1.8
PV3	53.53	50	1.0
PV4	114.89	74	1.5
PV5	58.54	63	0.92
PV6	111.56	63	1.77
PV7	39.61	36	1.1
PV8	126.26	21	6.01
PV9	15.54	17	0.91
PV10	14.5	111	0.13
PV11	78.99	58	1.36
total		529	1.10

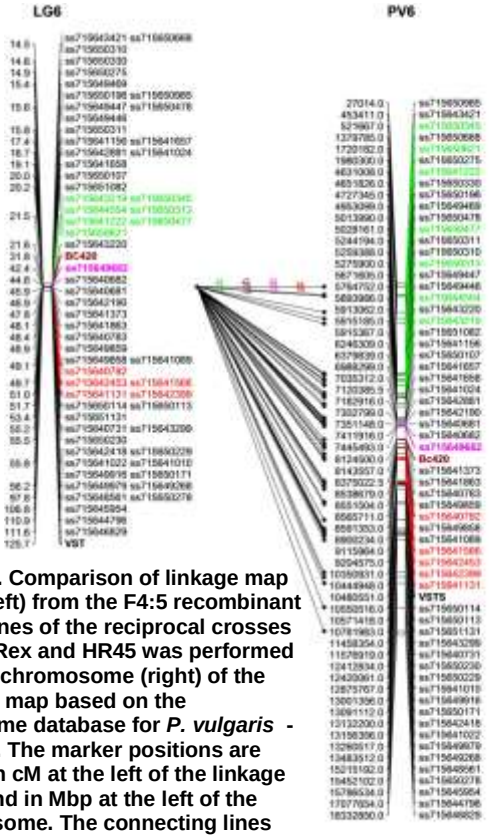

Figure 2. Comparison of linkage map group (left) from the F4:5 recombinant inbred lines of the reciprocal crosses of OAC Rex and HR45 was performed with the chromosome (right) of the physical map based on the Phytozome database for *P. vulgaris* - JGI v1.0. The marker positions are shown in cM at the left of the linkage group and in Mbp at the left of the chromosome. The connecting lines join homologous markers. QTL are at the left of the linkage group. QTLs are highlighted with red, green and purple colors. Markers linked to the BC420 QTL appear in bold red letters. BC42, a major QTL conferring CBB resistance. VSTS, color marker linked to the qBC420.

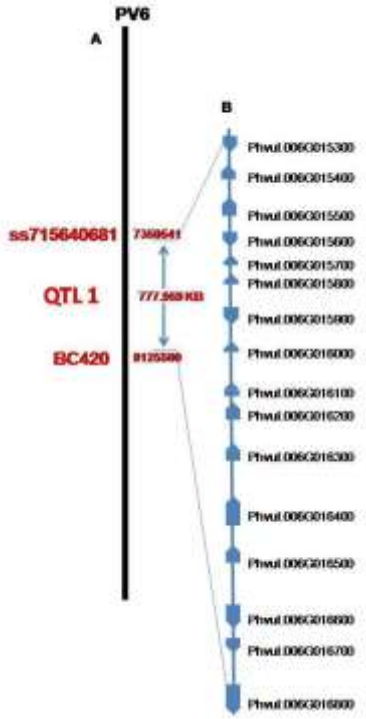

Figure 3. Candidate genes associated with CBB resistance in the QTL1 region. A, physical positions of main markers located around BC420 (in red). At left are listed markers and at right are physical positions in base pairs (bp). B, Predicted candidate genes for CBB resistance and locus names of candidate genes at right according to http://www.phytozome.net/.

Conclusion

genes were predicted to lie in the *qBC420* region and three of these predicted genes encode proteins involved in defense mechanisms against pathogens, including: CPK17 , RPM1- RIN4 and Myb. RIN4 is a well-known regulator of plant immunity (Liu et al., 2009) and may be a likely candidate for the resistance *R* gene itself.

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Environment

Keynote Speaker: Abstract

PULSES FOR HEALTHY SOILS, CROPS AND THE ENVIRONMENT

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The benefits of pulses to soil health, productivity and the environment can be categorized into N-related and non-N benefits. The N-related benefits hinge on their ability to fix atmospheric nitrogen, which means that pulses are grown with little or no fertilizer N. This benefit sometimes extends to nonlegume crops grown in rotation with pulses because they use some of the N recycled from decomposing pulse crop residues. The reduced fertilizer N requirements of pulse crops and subsequent nonlegume rotation crops means that healthy crops can be grown on these healthy soils with reduced fertilizer N inputs. The environmental benefits result from the reduced carbon footprint from the manufacture, transportation and application of fertilizer N, using less energy, thereby producing less greenhouse gases. In addition, less fertilizer N goes into the environment to pollute surface and ground waters. Pulse crop residues also improve soil biological health by promoting large, diverse soil microbial communities. These soil microorganisms add N to the soil through biological nitrogen fixation and/or cycling, resulting in reduced fertilizer N requirements. Diverse soil microbial communities also enhance biological disease and pest control, reducing the need for pesticides. The non-N benefits include residual soil moisture because pulses use less water than cereal crops, and they improve soil structure, thereby enhancing soil water and air movement. Pulses in rotations also break disease and pest cycles, reducing the need for pesticides. Therefore, growing pulses has soil, crop and environmental health benefits.

EO1

KEY FARMING TACTICS FOR LOWERING ENVIRONMENTAL FOOTPRINTS

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Intensified farming systems have been identified as a viable means to increase grain production. However, farming intensification requires more inputs such as fertilizers, pesticides, and fuels; all these emit greenhouse gases and have environmental consequences. An overwhelming question is: can farming practices be improved that enables yield increase with no cost to the environment? We reviewed 145 recent studies and identified some farming practices that can increase grain production while lowering the environmental footprint.

1. Reducing fertilizer use and including N_2-fixing pulses. Increasing N fertilizer has been shown to increase greenhouse gas emissions (**Fig. 1A**) and the carbon footprint (**Fig. 1B**) in oilseed and also cereal production. Furthermore, the emissions and carbon footprint were significantly influenced by N fertilizer applied to the previous crops, regardless of the different environments (**Fig. 2A**, wetter Indian Head and drier Swift Current), or different species (**Fig. 2B**). Including N_2-fixing pulse crops in a crop rotation allows the system to rely on the symbiotic N_2 fixation from the atmosphere, which significantly decreases the use of synthetic N fertilizer, thus lowering the carbon footprint (Gan et al. 2011).

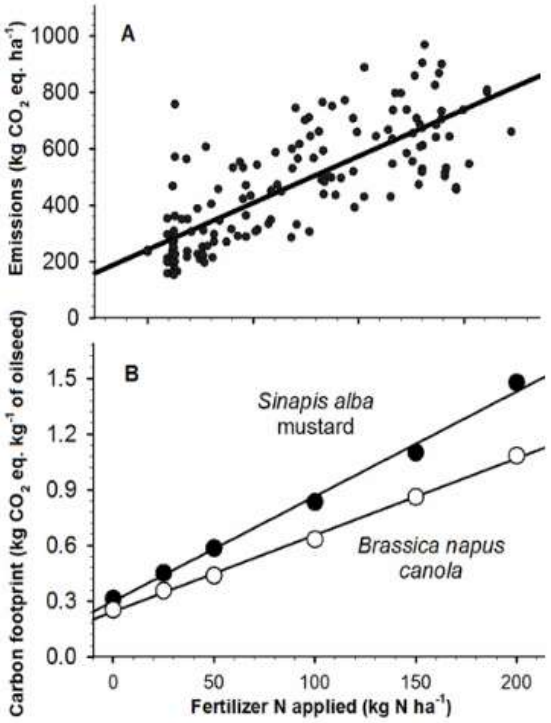

Fig. 1. Effect of N fertilizer on CO_2 emissions (A) and the carbon footprint (B) of the oilseed crops.

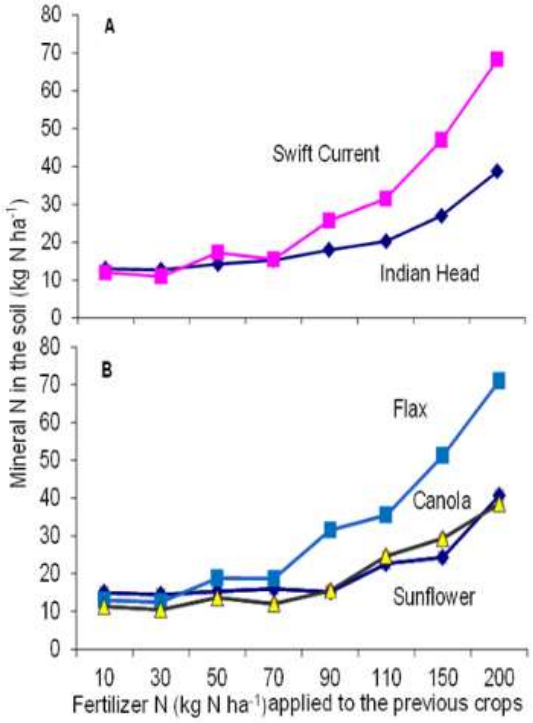

Fig. 2. Residual mineral N in the soil is a function of the N-fertilizer applied to the previous crops.

2. Diversifying crop rotations. Besides weed control, disease suppression, and production sustainability, crop diversification has also been considered a key cropping practice for improving agroecosystem productivity and lowering the carbon footprint (Gan et al. 2011) (Table 1).

Table 1. The carbon footprint of durum wheat grown in the various 3-yr cropping sequences at southwestern Saskatchewan, Canada.

Year1 - Year2 - Year3	Carbon footprint (kg CO_2 eq kg^{-1} of grain)		
	Mean[a]	Sdt Err	% decrease[b]
Cereal – cereal - durum	0.415	0.059	0
Cereal – oilseed - durum	0.375	0.059	-10
Cereal – pulse - durum	0.330	0.052	-21
Oilseed – cereal - durum	0.342	0.056	-18
Oilseed – oilseed - durum	0.316	0.051	-24
Oilseed – pulse - durum	0.295	0.049	-29
Pulse – cereal - durum	0.328	0.053	-21
Pulse – oilseed - durum	0.322	0.053	-22
Pulse – pulse - durum	0.273	0.045	-34

[a] Means of the 3 cycles of the 3-yr crop sequences at each of the two locations.
[b] Percent decrease compared with the cereal-cereal-durum monoculture system.

3. **Intensifying crop rotations.** Summerfallowing has long been used to conserve water and encourage the release of N in the soil. However, studies have shown that the frequency of summerfallow in a cropping rotation has a significant impact on the carbon footprint of the rotation (Schillinger et al. 2014). Crop intensification with reduced frequency of summerfallow in a rotation can increase crop production while reducing the carbon footprint.

4. Enhancing soil carbon sequestration. Greenhouse gas emissions associated with the crop production inputs can be offset by greater carbon conversion from atmospheric CO_2 into plant biomass and ultimately sequestered into the soil.

5. Manage tillage practices. Studies on how tillage affects the carbon footprint showed inconsistent results, varying with climatic conditions, soil type, and cropping systems. Reduced tillage combined with additional carbon input from cover crops or crop residue could improve the soil organic carbon content (Garcia-Franco et al. 2015).

6. Integrating agronomical practices. Many studies have shown that integration of agronomic practices can substantially increase crop yields without increasing or even decreasing greenhouse gas emissions.

In conclusion, meeting global food demand while lowering the environmental footprints can be achieved by adopting various improved agronomical practices, such as diversification of cropping systems; improvement of N fertilizer use efficiency; adoption of intensified rotation with reduced summerfallow; enhancement of carbon conversion from atmospheric CO_2 into plant biomass and ultimately sequestered into the soil; use of reduced tillage in combination with crop residue retention; integration of key cropping practices systematically; and inclusion of N_2-fixing pulses in crop rotations.

Gan et al. 2011 *Lowering carbon footprint of durum wheat by diversifying cropping systems. Field Crops Res. 122:199-206.*

Garcia-Franco et al. 2015 *Beneficial effects of reduced tillage and green manure on soil aggregation and stabilization of organic carbon in a Mediterranean agroecosystem. Soil Till. Res. 153:66-75.*

Schillinger et al. 2014 *Best Management Practices for Summer Fallow in the World's Driest Rainfed Wheat Region. Soil Sci. Soc. Am. J. 78:1707-1715.*

E02

BIOLOGICAL NITROGEN FIXATION BY SOYBEAN, PEA, AND LENTIL AND RECOVERY OF ABOVEGROUND RESIDUE NITROGEN IN THE SUBSEQUENT CROP IN SASKATCHEWAN, CANADA

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Estimating soybean N_2 fixation, partitioning of fixed N, and residue N recovery by following crops is important in assessing the impact of soybean [*Glycine max* (L.) Merr.] production on N budgets and cycling. The objectives of the research were to (1) use the ^{15}N dilution technique to estimate the N_2 fixation and the partitioning of fixed N between the grain and the straw of a short-season soybean in comparison to pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) under field conditions, and (2) to determine soybean above-ground residue N recovery by a following spring wheat (*Triticum aestivum* L.) crop at a site in the Black soil zone of Saskatchewan.

The grain N yield of soybean was lower than pea, while straw N yield was similar among the three crops. Percentage N derived from atmosphere (%Ndfa) was similar among the crops for both the grain and the straw, with soybean having relatively more even partitioning between the grain and the straw compared to pea and lentil. The majority of fixed N was retained in the grain, with 75% of total fixed N retained in the grain of soybean, 85% in pea, and 67% in lentil. The amount of biologically fixed N comprised over 70% of total above-ground N in soybean and over 60% in pea and lentil. In the subsequent year, wheat grown on soybean, pea, or lentil above-ground residues had similar N uptake in both the grain and the straw, while wheat grown on the wheat crop residue had lower grain N uptake. Similarly, percentage of N derived from previous above-ground (straw) residue N in either the grain (8-13%) or the straw (9-12%) of a following wheat crop was not different among the legume residues. The total amount of N derived from residue in wheat grain was greater for wheat grown on lentil residue (13 kg ha^{-1}) compared to pea (7 kg ha^{-1}) and wheat (3 kg ha^{-1}) residues.

Overall, residue N recovery rates in the grain and the straw of wheat in the subsequent year were similar for the different pulse residues, indicating similar N benefits to following crops from soybean, pea, and lentil. The substantial amount of N yield and N_2 fixation by short-season soybean as well as residue N recovery in the following crop imply a significant external contribution to the soil N budget and availability, similar to pea and lentil, suggesting promising prospects for soybean production to contribute to N fertility under Saskatchewan soil-climatic conditions.

EO3

BIOLOGICAL NITROGEN FIXATION BY PULSE CROPS ON THE SEMIARID CANADIAN PRAIRIE

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Pulses are a group of grain legumes grown worldwide and used as human food, animal feeds, and industrial demands. Pulses, like other legumes, have the ability to fix atmospheric N_2 and contribute to agriculture sustainability. Considering the importance of pulses in Canadian agriculture, we conducted this study to determine the effect of genotypic variability of various pulse crops on biological N_2 fixation (BNF) and the relationship between BNF and plant productivity in the semiarid Canadian prairies.

Materials and Methods

Field experiments were conducted at the Agriculture and Agri-Food Canada (AAFC) Research farm near Swift Current, Saskatchewan in 2008, 2009 and 2010. Treatments included eight market classes of the pulse crops namely chickpea, field pea and lentil, each with two or three cultivars, resulting in 23 species-cultivar combinations. In addition, one cultivar of each of dry bean and faba bean was included in the study. To quantify BNF by pulses, barley (AC Metcalfe) was used as reference crop. The experiment was arranged in a randomized, complete block design with four replicates. Standard plot management and plant protection measures were taken to raise a healthy crop. The BNF value was estimated using the ^{15}N isotope dilution method. For %Ndfa (percentage of N derived from the atmosphere) measurement, two micro-plots, 0.5 m × 4 rows, were established, one within each pulse plot and one in the adjacent barley pathway. A solution of 10 atom % ^{15}N-ammonium nitrate (4 kg ^{15}N ha^{-1}) was applied in the micro-plots at 3-4 leaf stage. Data were collected on above ground biomass and seed yield, N concentration in seed and straw. Data were analyzed using the Proc ANOVA in SAS9.3 (SAS Institute Inc., Cary NC).

Table 1: Seed and straw yield in various pulse crops

Pulses	Seed yield (kg ha^{-1})			Straw yield (kg ha^{-1})		
	2008	2009	2010	2008	2009	2010
Chickpea	2227 ± 88^{ab}	1520 ± 58^{b}	1263 ± 117^{c}	2675 ± 87^{c}	1667 ± 58^{bc}	4612 ± 178^{a}
Dry bean	870 ± 150^{c}	1057 ± 166^{c}	1902 ± 203^{b}	1740 ± 238^{d}	1263 ± 158^{c}	1713 ± 165^{c}
Faba bean	886 ± 132^{c}	1948 ± 264^{a}	3009 ± 343^{a}	2015 ± 217^{d}	2032 ± 225^{ab}	4634 ± 328^{a}
Field pea	2244 ± 108^{a}	2039 ± 68^{a}	2972 ± 100^{a}	4095 ± 154^{a}	2234 ± 70^{a}	3481 ± 96^{b}
Lentil	1904 ± 57^{b}	1272 ± 44^{bc}	2313 ± 90^{b}	3340 ± 78^{b}	1968 ± 71^{ab}	4196 ± 151^{a}
p-value	<.0001	0.0013	0.0001	<.0001	0.0022	<.0001

Data presented as mean ± standard error. Means with the same letters in a column are not significantly different; *p* represents the level of significance.

Results and Discussion

Pulses under study showed significant variation for seed yield in all three years, and in 2008, field pea showed highest yield of 2244 kg ha^{-1} followed by chickpea with 2227 kg ha^{-1} (Table 1). However, this trend did not continue for chickpea in either 2009 or 2010 but field pea had a trend similar to the previous year with a mean yield of 2039 kg ha^{-1} in 2009 and 2972 kg ha^{-1} in 2010.

Average yield of lentil was in the medium range over the years with 1904, 1272 and 2313 kg ha^{-1} in 2008, 2009 and 2010, respectively. Faba bean showed a huge variation for seed yield, was the lowest performer (886 kg ha^{-1}) along with dry bean (870 kg ha^{-1}) in 2008. But it produced highest quantity of seed in 2009 and 2010 accompanied by field pea, which was significantly higher than other pulses (Table 1). In general, seed yield of dry bean was lowest in this study although it did better relative to chickpea in 2010. In combined analysis over years, the pulses showed significant variation for seed yield and field pea appeared at the top of the table followed by faba bean (data not shown). Highly significant effect of growing year and year × species interaction on grain yield was also observed.

Table 2: Biological nitrogen fixation by various pulse crops

Pulses	%Ndfa			N fixed (kg ha^{-1})		
	2008	2009	2010	2008	2009	2010
Chickpea	39.22 ± 4.47^{b}	43.31 ± 3.42^{bc}	72.15 ± 2.17^{ab}	30.59 ± 3.63^{c}	21.02 ± 1.83^{bc}	103.59 ± 7.03^{a}
Dry bean	0.72 ± 0.11^{c}	32.45 ± 8.92^{c}	45.76 ± 7.8c	0.76 ± 0.32^{d}	15.37 ± 5.27^{c}	11.87 ± 2.23^{c}
Faba bean	54.34 ± 7.4^{a}	68.39 ± 4.17^{a}	79.72 ± 3.82^{a}	49.52 ± 3.64^{ab}	45.35 ± 10.9^{a}	107.69 ± 16.06^{a}
Field pea	43.29 ± 3.4^{ab}	56.23 ± 3.31^{ab}	62.33 ± 3.86^{b}	58.28 ± 6.15^{a}	36.59 ± 3.78^{ab}	68.5 ± 4.77^{b}
Lentil	41.5 ± 3.2^{ab}	50.59 ± 2.87^{b}	68.49 ± 3.04^{b}	38.24 ± 3.12^{bc}	23.03 ± 2.09^{bc}	86.76 ± 5.57^{ab}
p-value	0.0002	0.0022	0.0006	<.0001	0.0253	0.0004

Data presented as mean ± standard error. Means with the same letters in a column are not significantly different; *p* represents the level of significance.

The amount of N fixed by the symbiotic relationship between legumes and the soil rhizobial bacteria is determined by the relative dependence of the crop on biological nitrogen fixation for growth and the amount of N accumulated by the crop over the growing season. In our study, there were significant variations among pulse species for %Ndfa and BNF over the years (Table 2). The %Ndfa was generally lowest in 2008 and highest in 2010. In 2008, faba bean showed highest values and it was statistically similar to that of field pea and lentil (Table 2). In 2009, %Ndfa of faba and field pea was statistically higher than other pulses. Lentil and chickpea showed moderate %Ndfa. In 2010, faba bean also had highest %Ndfa but was statistically similar to that of chickpea. In combined analysis over years, the effect of growing year and pulse species on %Ndfa was highly significant ($p < 0.0001$). Pulses had a higher BNF in 2010 and lower in 2009. In 2010, faba bean had highest BNF of 108 kg ha^{-1}, followed by chickpea, lentil, field pea and dry bean of 104, 87, 69 and 12 kg ha^{-1}, respectively. The dry weather in 2009 reduced BNF which also impacted yield for chickpea and lentil. Among the pulses, field pea had most stable BNF with overall average of 55 kg ha^{-1}. Large effect of genetic variability in BNF and seed yield suggests the possibility that pulse cultivars with a high N_2-fixing ability and seed yield can be developed through selection for biological nitrogen fixation.

Conclusion

Field pea had consistent biological nitrogen fixation ability with stable and higher seed yield across years. It showed moderate tolerance to low rainfall induced drought stress in 2009 which suggest that field pea is more suitable for cultivation in the semiarid prairie than other pulses. The study highlighted the importance of environmental factors on BNF, particularly the negative effect of low rainfall on BNF and grain yield. Our results clearly show variation among pulse species in BNF and emphasize the importance of selecting appropriate species to exploit the potential of BNF in semiarid environment and develop a sustainable pulse production system.

EO4

ECONOMY WIDE ASSESSMENT OF PULSE REQUIREMENT IN 2030: CASE OF INDIA

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Pulses are essential source of protein, high in fibre and provide sufficient quantity of vitamins and minerals. Due to its large nutritional benefits for human health and soil fertility, the United Nations has proclaimed 2016 as the International Year of Pulses. Thus, an urgent attention is required to enhance the production of pulses not only to meet the dietary requirement but also to raise the awareness about pulses for achieving nutritional value, food security and environmental sustainability. Even though India is the world's largest producer (25%) and consumer (27%) of pulses, the country is importing a large amount of pulses (4.58 mt in 2014-15) from Canada, Australia and Myanmar to meet the growing domestic needs. The Government of India has launched a number of programmes for acreage, yield, productivity enhancement and plant protection centric improved technologies of pulse crops in India, however, not sufficient to meet the domestic demand. In this background, the current study makes an effort to evaluate the economic and environmental impacts of the targeted Pulse production in India in 2025 using Input-output framework. Further, it attempts to estimate the impact of pulse consumption according to required healthy diet guideline of India. A number of simulation exercises have also been attempted to study the implications of policies to reach the Pulse production target for India to feed the growing populations. Results show that the macro-economic impact of pulse production leads to increase in output and enhance soil fertility, however, the other agricultural sector makes necessary adjustments to meet the pulse target. The study also reveals a significant gap between the actual and recommended pulse consumption according to healthy diet guideline in India. Effective and continuous efforts are needed to increase the pulse production in India.

EP5

CONTROL OF SOYBEAN APHID BY PREDATORS PRESENT IN AGRICULTURAL LANDSCAPES IN MANITOBA

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The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a major invasive species in North America that causes significant yield losses in soybean production (Ragsdale *et al.* 2004; Heimpel *et al.* 2010). Since 2000, as a result of this new pest, soybean scouting services increased 40-fold and insecticide applications increased 130-fold (Tilmon *et al.* 2011). In Canada, soybean aphid was detected first in Ontario in 2001 (Hunt *et al.* 2003), and there were widespread population outbreaks in 2006 and 2008 in Manitoba (Gavloski 2006; Gavloski 2008). To manage soybean aphid outbreaks, it is important to determine factors regulating their populations in Manitoba, such as landscape complexity, and the abundance and patterns of movement of insect predators.

We assessed how agricultural landscape complexity and the patterns of predator movement affected soybean aphid suppression in 27 soybean fields in Manitoba over a two-year period. In each field, we infested ten potted soybean plants with aphids and exposed half of them to natural levels of predators and protected the other half using exclusion cages during a two-weeks period. Naturally occurring soybean aphids and predators were assessed collecting, five sweep-net samples (25 sweeps/sample) in each field studied each week of the experiment. We quantified landscape complexity at five different spatial scales (0.25, 0.5, 1, 1.5 and 2-km radius from focal soybean fields) using digital maps in ARC GIS 10 (ESRI 2010). Predator movement was quantified using 30 bi-directional Malaise traps located in the border between soybeans and neighboring habitats, including alfalfa, canola, wheat, grasses, and woodland. In addition, two mark-release-recapture experiments were conducted between soybean and alfalfa fields using seven-spotted lady beetles, *Coccinella septempunctata* (Linnaeus) (Coleoptera: Coccinellidae) in 2013 and 2014.

In all the fields and years studied, naturally occurring predators always suppressed aphids below the economic threshold level of 250 aphids/plant. On average, aphid populations protected from predators had a 3.79-fold increase compare to populations exposed to natural levels of predators. Naturally occurring soybean aphid populations were reduced (10.16 ± 2.42 aphids/25 sweeps, n = 225 in 2014 and 0.22 ± 0.12 aphids/25 sweeps in 2013). A total of 10,279 and 660 aphidophagous predators were captured in bi-directional Malaise traps and sweep-net samples respectively. Aphidophagous hover flies (Diptera: Syrphidae) were the dominant adult predators found in bi-directional Malaise traps (80% of the total capture) and sweep-net samples (36%), followed by minute pirate bugs (Hemiptera: Anthocoridae, 2 and 34%) and green lacewings (Neuroptera: Chrysopidae, 0.8 and 3.5%). Immature stages of minute pirate bugs (14%) were the dominant species found in sweep-nets, followed by hover flies (4.3%), damsel bugs (Hemiptera: Nabidae, 2.7%) and green lacewings (2.1%), brown lacewings (Neuroptera: Hemerobiidae, 0.6%) and lady beetles (Coleoptera: Coccinellidae, 0.2%). Soybeans (24.9 % of the total area, range 6.9 – 48.4 %), cereals (19.8 %, 2.9 – 46.9 %), canola (13.2 %, 0.0 – 36.2 %) were the major land-cover types in the landscapes studied. The proportion of cereals (wheat, barley, and oats) had a negative relationship with final aphid abundance whereas woodland and field-border grass showed positive associations with aphids. We found that the best predictors of aphid abundance were the levels of predator movement between soybean and neighboring fields, either alone or combined with independent landscape composition variables. Predators moved in greater numbers from woodland to soybean than vise versa, including lady beetles, aphidophagous hover flies, and minute pirate bugs, both

combined or separately. On the other hand, there was a trend of a greater number of predators moving from soybean to canola than the other direction, including aphidophagous hover flies and green lacewings. Also, lady beetles emigrated from alfalfa to soybeans. In the mark-release-recapture experiment, 36 seven spotted beetles (5.5 %) and 34 (5.6 %) were recaptured in 2013 and 2014, respectively. There was a trend of more beetles moving from soybean to alfalfa. In addition, beetle movement was faster and over longer distances in the same direction, perhaps due to the lack of soybean aphids in soybean fields and the abundance of aphids present in alfalfa fields (2.3 ±0.4 aphids/25 sweeps in 2013, 8.4 ± 0.2 aphids in 2014).

In this study, generalist predators exerted strong suppression of soybean aphid in different agricultural landscapes in Manitoba. In addition, predator movement between fields affects levels of pest suppression in soybeans, providing a mechanistic link between landscape complexity and ecosystem functioning. Furthermore, different crops and semi-natural land-cover types have distinct associations with pest suppression at the landscape scale, suggesting that grouping land-cover types into large categories (e.g. crop versus non-crop area) may not be appropriate for all systems or regions. Different spatial and temporal arrangements of crops could be used to increase the impact of predators in agricultural landscapes. Our results suggest that soybean fields located in landscapes with nearby cereals, alfalfa, and wooded areas may have better levels of aphid control by generalist predators. More studies are needed to determine the habitats used by generalist predators to reproduce and increase their populations before moving into soybeans.

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EP6

SOIL NUTRIENT SUPPLIES AND GREENHOUSE GAS EMISSIONS FROM TWO SASKATCHEWAN SOILS CONTAINING SOYBEAN, PEA, LENTIL, AND WHEAT RESIDUES

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While soybean acreage is expanding in western Canada, little is known about soil nutrient availability and greenhouse gas emissions in soils used for soybean production. An 8-week incubation experiment was conducted using intact cores collected in October of 2014 after harvest from two field trials containing different crop stubbles located near Saskatoon and Rosthern, Saskatchewan. The cores were frozen and stored for five months at -18^{o}C and then thawed prior to the incubation to simulate winter-spring conditions. We assessed soil available N and P supply rates, CO_2 and N_2O emissions, and $\delta^{15}N$-N_2O enrichment in soil cores from plots that had ^{15}N-labeled soybean, pea, lentil, and wheat residue.

Soil available N (NO_3^--N+ NH_4^+-N) and PO_4^{3-}-P supply rates decreased over the eight week incubation period in the Rosthern soil, with the supply rate of soil available N decreasing in the 5th week to about 25% of that observed in the first week. This pattern was consistent with the pattern of $\delta^{15}N$-N_2O enrichment, reflecting the depletion of mineralizable N released from added residue and soil, and a transition from labile fractions to more recalcitrant fractions at around the 5th week. The Rosthern soil amended with the soybean residue had more available P released than the pea or lentil residue at week 6, and more than the wheat residue at week 8. The Saskatoon soil with the lentil residue had higher N supplies from the second week till the end of the incubation. The CO_2 and N_2O fluxes did not differ significantly among different crop residue treatments in either soil, except that at the second week the CO_2 flux was higher in the Saskatoon soil amended with the soybean residue than with the pea residue. Similar patterns in nutrient release and greenhouse gas fluxes in the two soils with soybean, pea, or lentil residues suggest similar agronomic and environmental impact among the grain legumes grown under Saskatchewan conditions.

EP7

NODULATION PATTERN AND NITROGEN CONCENTRATION OF PULSE CROPS ON THE SEMIARID CANADIAN PRAIRIE

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Pulses play an important role in sustainable cropping system through symbiotic nitrogen (N) fixation. We studied the nodulation pattern and nitrogen concentration of selected pulse species, including chickpea, dry bean, faba bean, field pea and lentil as these traits are directly associated with N_2 fixation and protein content.

Materials and Methods

Field experiments were conducted at the Agriculture Agri-Food Canada Research farm, Swift Current, SK in 2008 - 2010. The experiments comprised eight market classes of pulse crops (field pea with green and yellow cotyledons, desi and kabuli chickpea, large green, small green, small red, and extra small red lentil), each with two or three cultivars. In addition, one cultivar each of dry bean and faba bean was also included in the study. The N concentrations were determined using an automated CN analyzer. The experiment was arranged in a randomized complete block design with four replicates. Number of nodules per plant was counted at early flowering and late flowering stages. Total number of nodules on the roots was counted and then dried for dry weight. At maturity, N concentrations in the pulse seed and straw were determined.

Results and Discussion

Pulses except dry bean had a higher number of early flower nodules in the wetter 2010 and lower in the drier 2009. In 2010, faba bean had highest number of early flower nodules followed by field pea and chickpea (Table 1). For late flower nodule number, chickpea had highest nodules in 2008 but was not significantly different from that of field pea and faba bean.
Seed as well as straw N concentration varied significantly over growing years (Fig. 1). Seed N concentration was highest in faba bean over the years followed by lentil. Faba bean also showed highest straw N concentration.

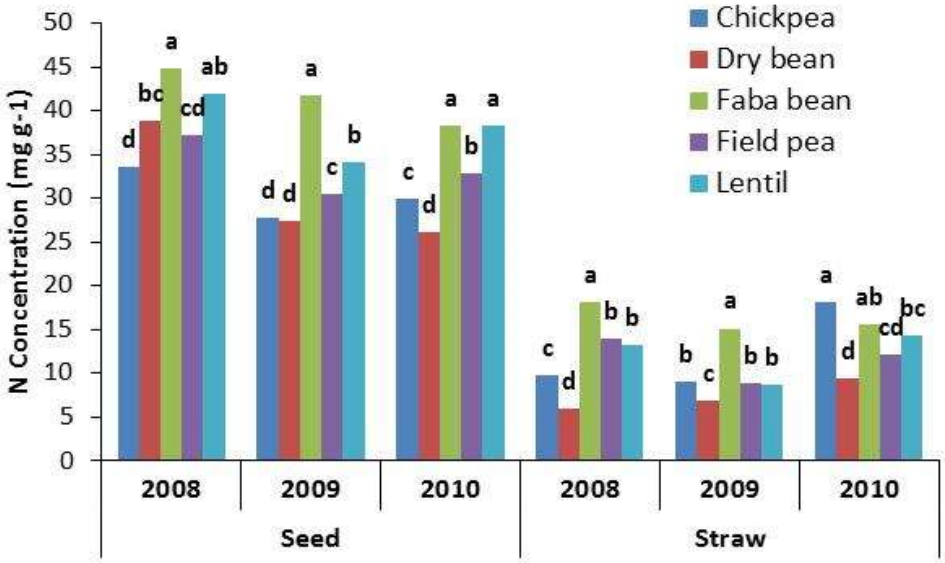

Fig. 1 N concentration in the seed and straw of various pulse crops.

Table 1 Nodulation pattern in different pulse species at early and late flowering stages.

Pulse	Early flower nodule number plant^{-1}			Late flower nodule number plant^{-1}	
	2008	2009	2010	2008	2009
Chickpea	25.86 ± 2.12^{a}	18.78 ± 3.29	37.86 ± 3.91^{b}	36.67 ± 2.83^{a}	15.41 ± 1.99
Dry bean	0.1 ± 0^{b}	8.3 ± 1.93	4.33 ± 1.58^{d}	0.87 ± 0.72^{b}	7.3 ± 1.96
Faba bean	35.2 ± 7.92^{a}	8.43 ± 4.78	50.27 ± 3.78^{a}	22.03 ± 14.14^{a}	7 ± 3.14
Field pea	28.2 ± 3.23^{a}	14.81 ± 2.78	40.72 ± 3.82^{b}	28.05 ± 2.45^{a}	13.51 ± 2.45
Lentil	21.69 ± 2.39^{a}	11.99 ± 1.53	24.01 ± 1.68^{c}	21.3 ± 1.18^{ab}	10.56 ± 1.39
p	*0.0064*	*0.1613*	*<.0001*	*0.0285*	*0.1043*

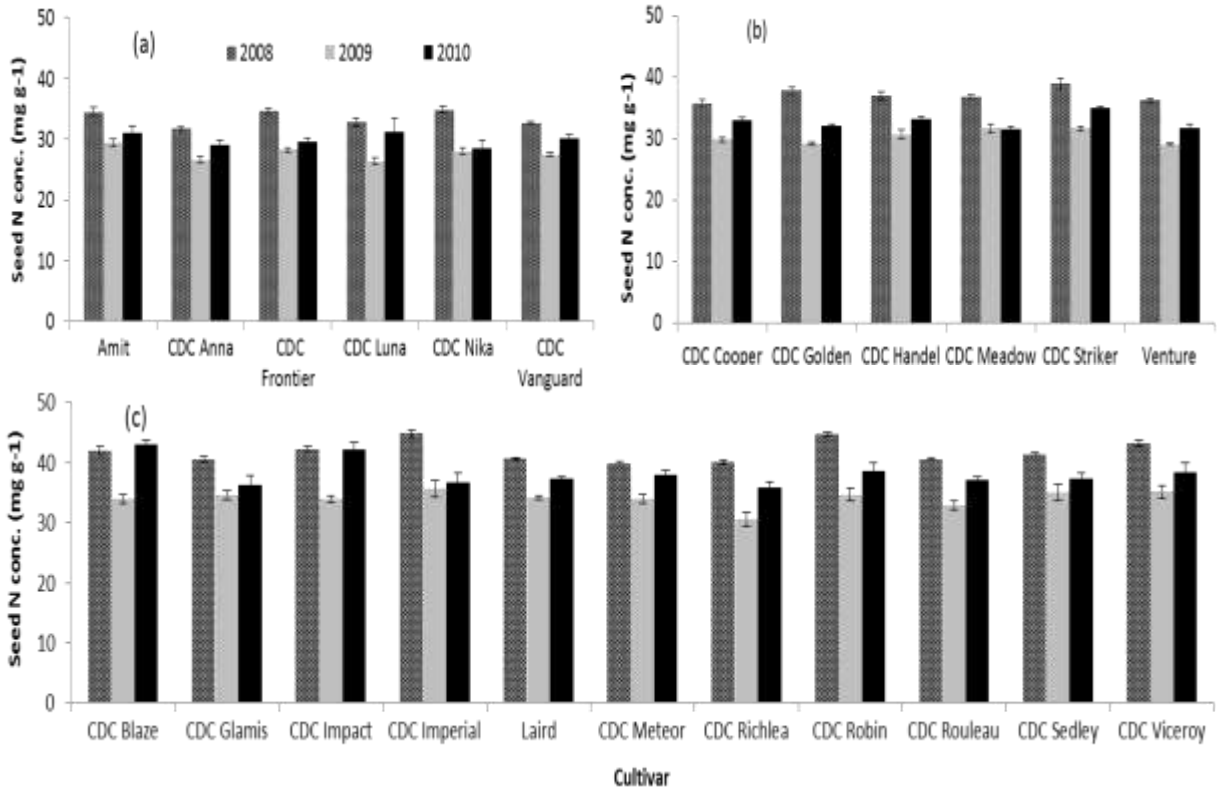

Fig. 2 Seed N concentration of chickpea (a), field pea (b) and lentil (c) cultivars in 2008-2010.

Conclusions

Number of nodules varied significantly among different pulse crops in most years. Faba bean had higher number of nodules across the year, followed by chickpea, field pea, and then lentil. N concentration in both seeds and straw varied significantly among different pulse crops in all the years. N concentration of field pea and lentil showed significant year × cultivar interaction. The study emphasized the importance of growing environment on nodule number and N concentration.

Agronomy and Pathology

Keynote Speaker- Abstract

ROOT ROT: AN ONGOING CHALLENGE TO PULSE PRODUCTION ON THE PRAIRIES

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When field pea, lentil and chickpea crops were first introduced onto the Canadian prairies in the 1970s and 1980s, they had relatively few disease problems. Unlike most foliar pathogens, the root rot complex was already present in Prairie soils, but has increased over time with repeated pulse cultivation. The root rot complex on pulses includes *Fusarium* spp., *Pythium* spp., *Rhizoctonia solani* Kühn and *Aphanomyces euteiches* Drechs. *Aphanomyces euteiches* has (likely) always been present in the Prairie region, but has recently been identified as the cause of severe damage in pea and lentil. In 2014-2015, *A. euteiches* was present in 40-50% of surveyed fields across the region. It is favoured by wet soils, but can cause injury under normal conditions when inoculum levels are high. Most of the pathogens in the root rot complex can be reduced using complex cocktails of seed treatments together with a 4-year crop rotation. However, no fungicides effective against *A. euteiches* have been identified, and normal cropping rotations are not very effective in reducing inoculum levels. No strong sources of resistance have been identified, but studies to identify the genes involved in partial resistance / tolerance are in progress. At present, the only reliable response to this pathogen is to avoid heavily infested fields. Molecular methods are being developed to rapidly identify fields with high levels of inoculum, and marker-assisted selection techniques are being developed to speed the selection of partially resistant cultivars.

PO1

PEA SEED-BORNE MOSAIC VIRUS (PSbMV) SEED TRANSMISSION IN FIELD PEA

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The potyvirus *Pea seed-borne mosaic virus* (PSbMV) is non-persistently transmitted to field pea (*Pisum sativum* L.) by aphids. Losses to PSbMV can be substantial and are influenced by host genotype, infection frequency, and virus pathotype. The objectives of this research were to evaluate field pea cultivars grown in North Dakota, USA for resistance to PSbMV and determine the rate of viral seed transmission. Isolate ND14-1, recovered from field pea seed produced in North Dakota, was determined to be 99% identical to a pathotype 4 (P4) PSbMV via whole genome sequencing. Twenty field pea cultivars were mechanically inoculated 5 weeks after planting (WAP) with ND14-1. PSbMV infection frequency was evaluated using enzyme-linked immunosorbent assay (ELISA). Two cultivars contain the sbm-1 gene and were resistant to ND14-1 PSbMV infection. One cultivar was moderately susceptible (24%), and all other cultivars were susceptible (50-77%). Weight reductions in daughter seed from infected plants harvested at 15 WAP ranged from 0-52.4% with increases in seed number ranging from 0-37.7%, compared to non-inoculated plants. Transmission in daughter seed from PSbMV positive plants ranged from 0-60%, depending on cultivar. Seed transmission did not differ significantly in any cultivar based on the presence of seed symptoms.

PO2

OCCURRENCE AND DISTRIBUTION OF PLANT-PARASITIC NEMATODES IN PULSE CROP FIELDS OF WESTERN CANADA

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1. Introduction

Plant and soil nematodes are small (<4mm long) roundworms in which some species are economically important as pest of a variety of crops. Plant parasitic nematodes are responsible for over $100 billion in annual crop losses worldwide. Despite this, very few studies have investigated the current distribution and economic importance of plant parasitic nematodes for pulse crops in the prairies, which include major export crops for Canada. The majority of previous studies examining nematode communities was performed several decades ago, and may now be suspect as a result of recent molecular identification methods (as has been the case with the quarantine pest *Ditylenchus dipsaci*). The quarantine pest nematode, *Ditylenchus dipsaci*, can hamper securing export markets for some crops, it has been particularly problematic for yellow pea exports from Canada to India. However recent molecular testing has revealed that previous identifications of *D. dipsaci* in yellow pea exports have actually been the non-quarantine species *D. weischeri*. In other pulse regions, nematodes are economically significant pests of lentils, pea, chickpea, and faba bean. To further our understanding of these issues, a survey was conducted in the Prairie Provinces to study the occurrence of plant-parasitic nematodes associated with pulse crops.

The objectives of this study were:

- Conduct a survey in three major pulse crops growing regions in Canada (Alberta, Saskatchewan and Manitoba) to study the occurrence of plant-parasitic nematodes associated with lentil, pea, chickpea and faba beans, including the quarantine pest *Ditylenchus dipsasi*.
- Help resolve the long-standing market access issue of yellow pea exports to India and address the gap in understanding of the distribution of plant-parasitic nematodes of pulse crops in Prairie Canada
- Improve awareness for proactive control

2. Materials and Methods

A total of 366 soil and plant samples of yellow pea, chickpea, lentil and creeping thistle from 103 fields have been examined. The fields were visited at the mid-reproductive growth phase (R4 or R5) to maximize the likelihood of recovering foliar nematodes such as *Ditylenchus*. Nematodes were extracted following standard sieve methods for leaves, stems, and seeds separately. Soil nematode extractions followed the sugar flotation method. Nematodes were identified to genus level by morphological features and at the species level using molecular analysis (species-specific PCR, PCR-RFLP, ITS sequence of rRNA gene).
In order to purify the DNA, PCR products of interest for purification were purified using with protocol following the manufacturer's instruction. Samples were sent for sequencing for later identification by sequence comparison of the ITS1+ITSII region of the 18s rRNA gene in Genbank.

3. Results and Discussion

Table 1: Number of positive fields of plant parasitic nematode taxa found in Manitoba, Alberta and Saskatchewan in 2014 and 2015.

	Number of Positive Fields*			
Nematode	Above Ground Crop	Above Ground Weed	Soil Crop	Soil Weed
Aphelenchoides spp.	13	2	33	31
Aphelenchus spp.	16	1	12	8
Ditylenchus spp.	3	2	9	7
Ditylenchus dipsaci	1	–	–	–
Ditylenchus weischeri	1	11	–	–
Helicotylenchus spp.	–	–	15	11
Hoplolaimus spp.	–	–	1	1
Longidorus spp.	–	–	0	1
Merlinius spp.	–	–	1	0
Paraphelenchus spp.	–	–	1	1
Paratrichodorus spp.	–	–	–	1
Paratylenchus spp.	–	–	26	21
Pratylenchus spp.	–	–	13	6
Subanguina spp.	2	–	1	0
Tylenchorhynchus spp.	–	–	32	33
Xiphinema spp.	–	–	3	3

* Data from plants and soil from 2015 survey (270 samples analysed) and soil from 2014 survey (96 samples analysed). Plant samples results from 2014 survey are not included in the table.

Fourteen genera of plant-parasitic nematodes have been recovered from soil and (or) plants of peas, chickpeas, lentils and thistle plants from the Canadian Prairies (Table 1).

Aphelenchoides, *Tylenchorhynchus* and *Paratylenchus* were the most frequently encountered genera of plant parasites. *Pratylenchus spp*. also occurred commonly, and two fields had levels high enough (1280 and 1760 nematodes per nematodes/kg soil) to indicate a potential nematode problem. These two fields were positive for *Pratylenchus neglectus* according to molecular tests.

D. dipsaci was recovered from one yellow pea field in Manitoba and *D. weischeri* from twelve fields in Manitoba and Alberta, mostly from thistles samples. *D. dipsaci* has recently been reported in two garlic fields in southern Manitoba and *D. weischeri* is not considered an agricultural pest. Molecular identification of nematodes is still in progress and sampling from 2016 has just been completed.

PO3

QUANTIFICATION OF *FUSARIUM AVENACEUM* IN SOIL AND CROP RESIDUES FROM PEA FIELDS IN ALBERTA

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Introduction: Pea root rot is a complex disease caused by a combination of pathogens such as *Fusarium* spp., *Aphanomyces euteiches*, *Pythium spp.* and *Rhizoctonia solani.* Root rot can cause significant yield losses which may limit pea production. Among the Fusarium species involved in pea root rot in western Canada, *F. avenaceum* is the predominant species[1, 2]. There is no complete source of resistance to *F.avenaceum* in field pea however partial resistance has been reported. Accurate detection and identification of associated pathogen is an essential step in disease control and choosing management strategies. One of the methods used for this purpose is quantitative PCR (qPCR), which provides reliable and accurate information about quantity of pathogens which can be used for predicting the disease risk base on initial inoculum. The aims of present study were to: 1) design and develop specific and sensitive DNA-based assay for quantification of *F.avenaceum*, 2) understand the relation between *F.avenaceun* DNA levels and disease severity under controlled condition, and 3) use the assay for quantification of pathogen in soil and crop residues collected from commercial fields in Alberta to estimate pathogen population.

Materials and Methods: Specific primers and probe were designed for *F. avenaceum* based on partial translation elongation factor alpha 1 (TEF-1α) gene sequences obtained from NCBI. The candidate assays were then tested for their specificity against other Fusarium species and other common soil-borne fungi, and reaction parameters to optimize sensitivity were tested. In order to correlate disease severity to inoculum levels trials were conducted under greenhouse condition using two inoculation methods. For preparing macroconidia inoculum, mycelia were incubated in carboxymethyl cellulose (CMC) media on a shaker at 1400 rpm for 7 days. The macroconidia was then diluted to provide concentrations ranging from 10^6 to 10^2 macroconidia/g soil. For the second inoculation method, *F. avenaceum* was grown on autoclaved wheat stubble, dried, ground and mixed with Cornell mix to provide concentrations ranging from 20 to 0.5% v/w. Pea seeds ('CDC Meadow') were surface sterilized, planted into each soil inoculum level, and roots rated after 4 weeks on a 1 (healthy) to 7 (dead) point scale. Soil samples were removed prior to planting and kept for qPCR analysis. In order to test the assay for samples with natural inoculum, soil and crop residues from commercial fields were collected before planting and during the flowering stage. 10 samples were collected from each field; 5 each from sites with and without disease symptoms. DNA was extracted from the samples using the MoBio PowerSoil DNA isolation kits and used in the qPCR assay developed above.

Results: A specific and sensitive real time assay was developed and optimised for identification and quantification of *F.avenaceum*. Testing against several *Fusarium* species and other common soil-borne fungi indicated that the designed primer/probe set was highly specific without cross-amplification of other Fusarium species and soil-borne fungi. Results indicated that low concentrations of *F.avenaceum* can be quantified by using these primers and probe. A linear relationship was observed between the amount of pathogen inoculum, root rot severity and CT value (Figure 1). Comparing the rate of disease severity between two inoculation methods indicated that inoculum on wheat stubble caused higher disease severities than conidia inoculum. Quantification results from samples collected from fields indicated that quantity of *F.avenaceum* detected in soil samples was low; however the amount detected in previous crop stubble and pea stubble was

relatively higher (Table 1). Amount of initial inoculum has an important role in disease progress. Results suggest that it is important to improve crop residue management since *F.avenaceum* survival is increased in crop residues compared with soil. The outcomes from this study, combined with additional information about other pathogens involved in root rot disease and environmental parameters, will be applied toward developing a disease risk model. The tool should be validated in field conditions, and among different soil types and environmental conditions, for use in predicting disease risk before planting pea.

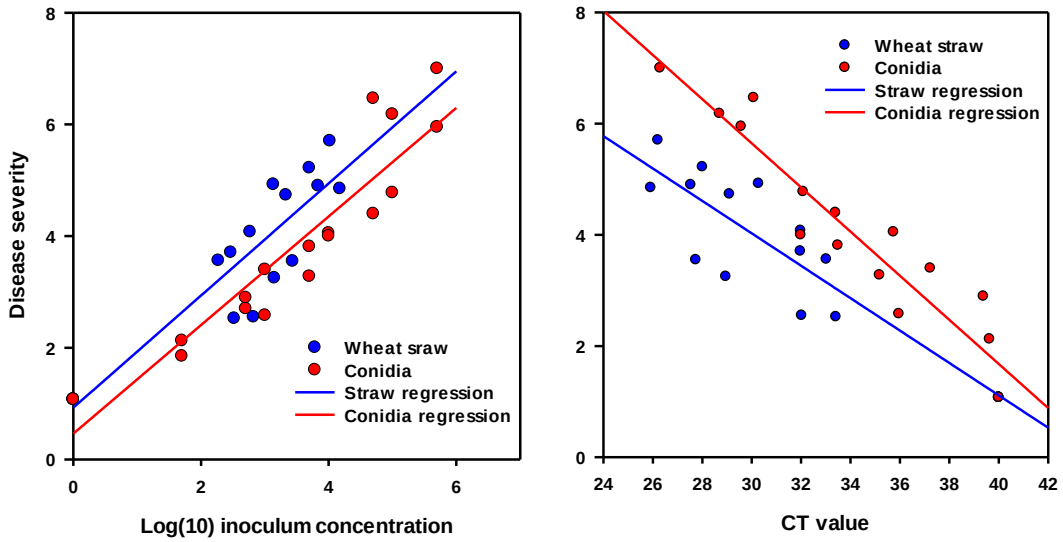

Figure 1. Linear correlations between *F. avenaceum* inoculum dose (conidia or mycelia fragments), disease severity (left) and CT value (right).

Table 1. CT value, measured by quantitative PCR using primer/probe set designed for *F. avenaceum,* from previous crop stubble and pea stubble collected during flowering and post-harvest, respectively.

Field	Previous crop stubble	Pea stubble
1	26.8	27.1
2	31.5	28.3
3	30.6	25.5
4	29.1	25.0
5	29.7	25.5

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PO4

CAUSAL AGENTS OF NECROTIC SPOTS ON FABA BEANS: LYGUS, *BOTRYTIS* SPP. OR BOTH?

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Introduction: Faba bean (*Vicia fabae* L.) is an important grain legume with high protein content and biomass. It is a high nitrogen fixing, lodging resistant, cool weather and moisture loving crop that can attain high yields in Alberta and Saskatchewan. Faba bean seed quality and marketability is often downgraded due to the presence of necrotic spots and hull perforations on the seeds. The upper limit of perforated damage acceptable for faba beans to be graded 'No. 1 Canada' is 1% as per the Canadian Grain Commission guidelines (Canadian Grain Commission, 2016).

Objectives: Determine the potential field association between chocolate spot and lygus bugs in faba bean and quantify joint damage to faba bean seeds.

Hypothesis: *Botrytis* spp., cause of chocolate spot (CS) disease, and lygus. are often found concomitantly in faba bean fields. Due to the fact that CS develops during the flowering stage and lygus feeds on the flower buds and pods, it becomes difficult to explain the primary cause of necrotic spots and how these affect faba bean seed quality.

Methodology: In 2015, field surveys were performed in commercial faba bean fields in Alberta at the pod stage to evaluate the relative impact of Botrytis infection and lygus feeding on the severity of necrotic spots on faba bean seeds. Seed damage was quantified by visual assessment and fungi colonizing the seeds were determined by plating on potato dextrose agar. Correlation analysis among insect abundance, CS disease severity measured by assessing the plant foliage, and faba bean seed damage was conducted to test for possible associations.

Outcome: CS severity was generally low while lygus abundance ranged from 6-27 lygus per 10 sweeps (Fig.1). Seed damage was significantly higher for seeds collected from the top of the plant, where lygus bugs were more abundant than the middle or bottom of canopy where CS was more severe. CS disease severity had no direct correlation on the seed severity, however positive and very moderate correlation was observed between the lygus abundance and seed severity (Fig. 2 & 3). This suggests lygus feeding on pods causes the development of necrotic spots on faba bean seeds. Despite low CS disease severity, *Botrytis spp.* were frequently isolated from seeds (Fig. 4). The highest frequency (10 - 21%) of isolation was observed from Lacombe, Parkland, Starland and Wetaskiwin which also had relatively high numbers of lygus abundance.

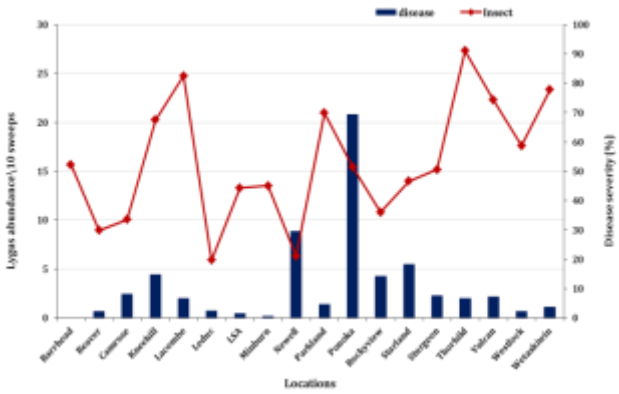

Fig. 1 Distribution of Lygus and chocolate spot on faba beans in west central

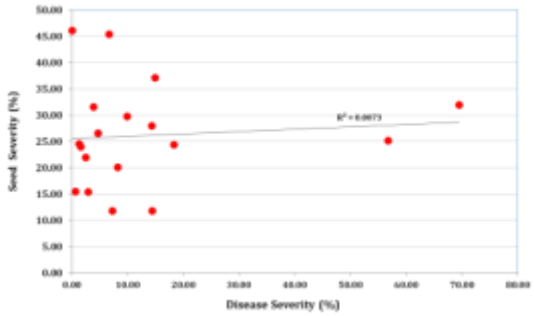

Fig. 2 Correlation between seed severity (%) and CS disease

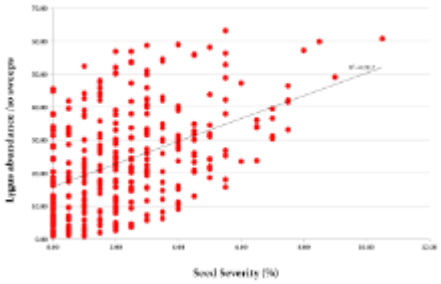

Fig. 3 Correlation between seed severity (%) and lygus

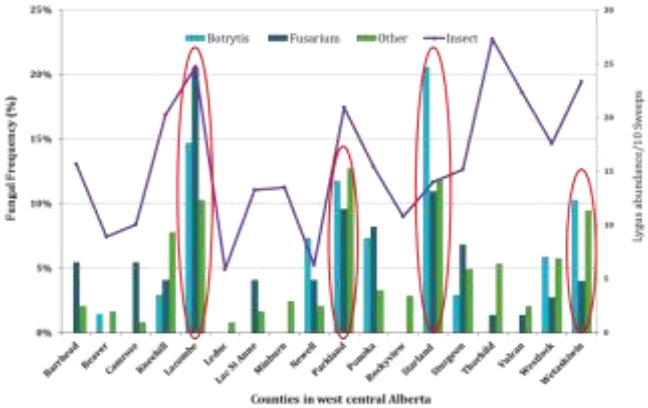

Fig. 4 Lygus abundance and isolation frequency of Botrytis and other saprophytic fungi isolated

Conclusion: The apparent seed quality loss caused by necrotic spots could be attributed to the mechanical and physiological injury caused by lygus feeding on the faba bean seeds allowing an entry point for Botrytis colonization, as evident from the high seed infestation. Greenhouse trials to further investigate this potential interaction are underway.

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PO5

RISK ASSESSMENT AND MANAGEMENT STRATEGIES TO PREVENT ROOT ROT OF FIELD PEA CAUSED BY *APHANOMYCES EUTEICHES* IN ALBERTA

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Abstract

Aphanomyces root rot (ARR) of field pea, caused by *Aphanomyces euteiches*, can result in severe root damage, wilting and large yield losses in rainy years. To explore ways to manage this disease, the effect of *A. euteiches* inoculum density on field pea was studied under greenhouse and field conditions in 2015 and 2016. Increasing inoculum density adversely affected seedling emergence and plant vigour, and resulted in increased root rot severity in both the field and greenhouse. The fungicidal seed treatments Apron Advance, Intego Solo, BAS 516F, BAS 720F and BAS 516F + BAS 720F were evaluated for their efficacy against ARR. All seed treatments except Apron Advance reduced root rot severity in the field and greenhouse. BAS 516F, BAS 720F and Intego Solo improved plant vigour and all treatments prevented seedling blight to varying degrees under greenhouse conditions. A collection of 22 pea genotypes was evaluated for resistance to root rot in field plot experiments. The line 00-2067 exhibited the highest level of resistance to *A. euteiches*. This study demonstrated the potential effectiveness of fungicidal seed treatments and partial host resistance to manage ARR of field pea.

Introduction

Field pea (*Pisum sativum* L.) is a valuable cash crop with high protein content and the ability to improve the N balance in the field (Hossain et al. 2014). Unfortunately, pea cultivation is limited by Aphanomyces root rot (ARR) caused by *Aphanomyces euteiches*, the most destructive soil-borne pathogen of field pea in Alberta **(**Chatterton et al. 2015). This parasite was first described by Jones and Drechsler (1925), and outbreaks of ARR have been reported recently in Alberta (Chatterton et al. 2015, Hwang and Chang 1989). According to Pfender and Hagedorn (1983), *A. euteiches* can cause yield losses of field pea up to 86% in heavily infested crops. The longevity of the pathogen oospores limits the utility of traditional crop rotation as a strategy to manage ARR, while seed treatments and cultivar resistance have not been evaluated as control measures in Alberta. The objectives of this study were to examine the impact of *A. euteiches* inoculum density on ARR severity, and evaluate the effectiveness of seed treatments and genetic resistance as root rot management tools in field pea.

Materials and Methods

Inoculum density experiments

Field plots were established on June 15, 2015 and May 17, 2016, in Edmonton, AB, to determine the effect of inoculum density on seedling growth and productivity. The seeds were sown at a depth of 5 cm with a push seeder and mixed with sand inoculum at a concentration of 150 *A. euteiches* oospores/mL, as well as with a commercial rhizobial inoculant (10 mL/6-m row). Four inoculum concentrations (0, 0.3×10^2, 6×10^2 and 1×10^3 oospores/row) were applied. The trial was arranged in a randomized complete block design (RCBD) with four replicates. Emergence counts and seedling

vigor (0-4) were recorded at 2 and 4 weeks after seeding, respectively. Root rot severity was recorded for 10 plants, taken randomly from each plot, on a scale of 0-4 (healthy to dead). The plots were harvested on September 29, 2015 and September 7, 2016 and the seeds were weighed to determine yield. In a greenhouse experiment, the pea cultivars Abarth and Horizon were sown into 600 mL plastic cups containing 400 mL Pro Mix potting medium, 100 mL *A. euteiches* inoculum and 1 mL rhizobium. The seeds were planted at a depth of 2 cm and density of 10 seeds per cup, and the sand inoculum included various concentrations of *A. euteiches* (720 colony forming units (CFU)/mL, 360 CFU/mL, 180 CFU/mL, 90 CFU/mL, 45 CFU/mL, 22.5 CFU/mL, 11.2 CFU/mL, 5.6 CFU/mL, 2.8 CFU/mL or a pathogen-free (0 CFU/mL) control). The cups were arranged in a RCBD with 6 replicates per treatment. The emergence rate, height and vigour (0-4) of seedlings were measured at 7 days after seeding. Root rot severity and nodulation were evaluated on a scale of 0-4 in the third week after seeding. The experiment was repeated.

Fungicide seed treatment experiments

A field experiment was established on June 15, 2015 and May 17, 2016, at Edmonton. Seed of the susceptible cv. Horizon was coated with one of five fungicide formulations, and sown along with 150 mL sand inoculum (350 CFU/mL) per 6-m row. Untreated seed was sown in inoculated and non-inoculated controls. Data collected included emergence rate, root rot severity, vigor and nodulation rates. Plots were harvested on September 29, 2015 and September 7, 2016, and yield was determined. The same seed treatments also were evaluated in a greenhouse experiment with two susceptible pea cultivars, Horizon and Abarth, with treatments arranged in a RCBD. Six replicates were included per treatment and the experiment was repeated. The inoculum concentration was 360 CFU/mL in the greenhouse experiments. The emergence rate, height and vigour of the seedlings, as well as root rot severity and nodulation, were measured as above.

Evaluation of cultivar resistance/tolerance

A total of 22 field pea genotypes were evaluated for their response to *A. euteiches* under field conditions in Edmonton, AB. Treatments were arranged in a randomized split-plot design with 4 replicates, and were seeded on May 2015 and 2016. Sand inoculum of *A. euteiches* (350 CFU/mL) was applied at a rate of 150 mL per row. Emergence rates, root rot severity, vigor and yield were measured as described above. The plots were harvested on September 23, 2015 and September 8-9, 2016. A GGE biplot analysis was carried out to investigate the stability of resistance to *A. euteiches* and yield performance of the pea genotypes over the field trials.

Result and discussion

Inoculum density

Under field conditions, all of the inoculated treatments had a higher ARR severity and lower pea germination and vigour compared with the non-inoculated controls. Germination and seedling vigor decreased with increasing inoculum concentration. A linear relationship was observed between root rot severity and emergence rate, nodulation and vigor. In the greenhouse experiment, as the inoculum density increased, pea root rot became more severe, resulting in reduced plant height and nodulation. Root rot severity increased quickly at *A. euteiches* concentrations of 11.3 CFU/mL or greater, indicating that outbreaks of ARR may require a threshold level of pathogen inoculum in the soil. A linear relationship was found between nodulation and root rot severity, suggesting that *A. euteiches* infection could suppress nodule formation.

Fungicide seed treatment

In the field experiment in 2015, symptoms of root rot were first observed as a brown discolouration 4 weeks after seeding. None of the treatments significantly improved emergence or seedling vigour relative to the inoculated control. Nonetheless, disease severity was lower following treatment with Intego Solo, BAS 516F, BAS 720F or BAS 516 + BAS 720 compared with the inoculated control.

Nodulation was greater relative to the control when seeds were treated with Apron Advance. None of the treatments improved yield, although late seeding and dry summer weather may have also contributed to poor yield. It is possible that soil conditions and other soil microorganisms also could impact the severity of ARR, but this was not examined. The results of the greenhouse study indicated that seed treatment with Intego Solo resulted in the greatest mean height, vigour and nodulation, and the lowest root rot severity. Treatment with BAS 516F and BAS 720F also resulted in improved growth parameters and reduced disease severity.

Resistance/tolerance to ARR

Root rot severity in the inoculated and non-inoculated plots was significantly different for all the pea genotypes examined. Diseases severity was lowest in line 00-2067, which was previously reported to be partially resistant to ARR (Conner et al. 2013). The cv. Saffron had the highest yields in both the non-inoculated control plots and the inoculated plots. The GGE biplot analysis indicated that line 00-2067 was the most resistant and stable genotype, while cv. Spring D had the lowest percentage reduction in yield. The variance observed in various parameters across years and sites, including for root rot severity and percentage yield reduction, may have reflected different weather conditions, particularly with respect to the amount of rainfall, in the two years of the study.

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PO6

INTRA-HOST INTERACTIONS OF SOIL-BORNE PATHOGENS AND AN INSECT HERBIVORE IN FIELD PEA

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Introduction:

Considerable increases in land area devoted to pea production combined with short cropping intervals has led to a higher incidence and severity of root rot disease and the range expansion of the pea leaf weevil (*Sitona lineatus*) into the Prairie provinces. There are currently no satisfactory management strategies available to address either of these constraints to pea production. *Fusarium* spp. and *Aphanomyces euteiches* are among the most prevalent and damaging pathogens within a complex of microbes known to cause root rot disease. Pea leaf weevil larvae also cause damage to roots by feeding on *Rhizobium* root nodules, reducing yield volume and quality by impairing nitrogen fixation. There is a high probability that these organisms interact synergistically, increasing disease severity and yield loss. Identification of these interactions will be the first step in the design of effective mitigation strategies.

Research Objectives:

1. Assess differences in symptom severity and insect survival following exposure of pea to root pathogens and pea leaf weevil (PLW) in a controlled greenhouse environment
2. Test the efficacy of seed treatments in mitigating the detrimental effects of soil-borne pathogens and PLW on plant development and yield in the field

Materials and Methods:

Root rot severity was assessed using a disease severity index, ranging from 0 (healthy plant) to 5 (100% decay, plant is dead). Additional health parameters measured included root fresh weight, shoot height, counts of feeding notches and PLW present in soil and root nodules.

Greenhouse Experiments

Pea plants were grown from seed in nitrogen-deficient soil inoculated with *Rhizobium leguminosarum* to facilitate nodulation, and were exposed to *F. avenaceum* and PLW either in isolation or combination. Soil was inoculated with *F. avenaceum* at the time of seeding, and PLW eggs were added two weeks after planting. Individual pots were sealed into mesh cages and allowed to grow until the plants began to flower, at which time health parameters were assessed. Each treatment was replicated six times.

Field Trials

Pea and faba bean (*Vicia faba*) were grown in three locations in southern Alberta. Seeds were treated with fungicides, insecticides, or both (Table 1). Five plants per plot were randomly sampled over three sampling periods, occurring at the 10th node, pre-flowering and late flowering/early pod formation stages. Each treatment was replicated in six plots.

Table 1. Experimental treatments to test the efficacy of fungicidal and insecticidal treatments	
1	Check
2	Fludioxonil **(Apron)**
3	Fludioxonil + ethaboxam **(EthAp)**
4	Thiamethoxam **(ThiSd)**
5	Fludioxonil + ethaboxam + thiamethoxam **(ThiSdAE)**

Results and Discussion:

Greenhouse Trials

Simultaneous exposure of pea to *F. avenaceum* and *Sitona lineatus* significantly increased disease severity in comparison to plants exposed only to *F. avenaceum* (Figure 1). Insect survival was also significantly increased when *F. avenaceum* was present, likely due to an increase in nodule production as a compensatory response (Figure 2).This indicates that interactions are occurring that increase both disease severity and insect fitness.

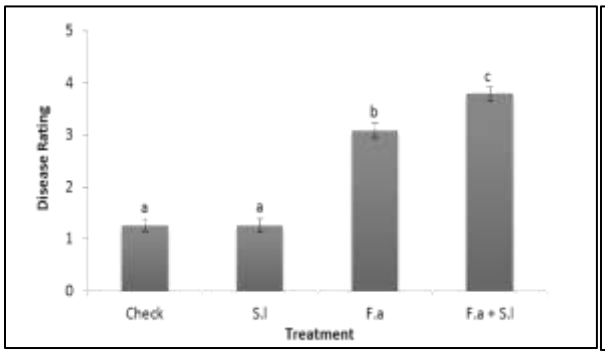

Figure 1. Average disease rating by treatment

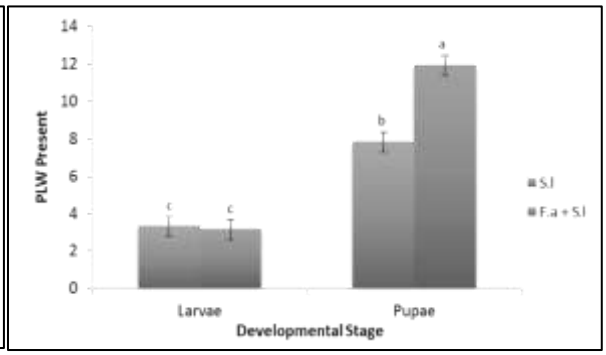

Figure 2. PLW survival by treatment

Field Trials

The fungicides fludioxonil and ethaboxam showed variable success in controlling root disease, with significant effects occurring only in early stages of plant development (Figure 3). Thiamethoxam was effective in controlling pea leaf weevil herbivory, and this effect persisted into the early flowering stage and when combined with fungicides (Figure 4). These results indicate that avoidance techniques and long cropping intervals of susceptible crops remain the only effective means of controlling root rot-related yield losses.

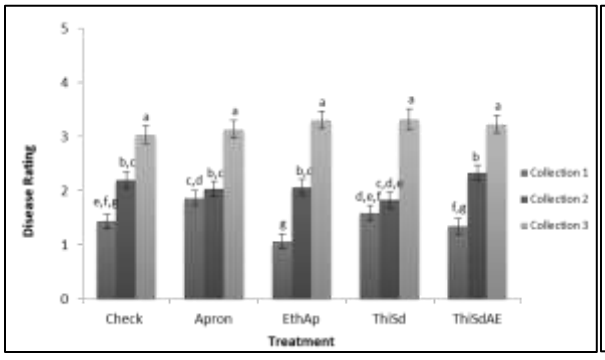

Figure 3. Disease ratings over three sampling periods in plants grown from treated seed.

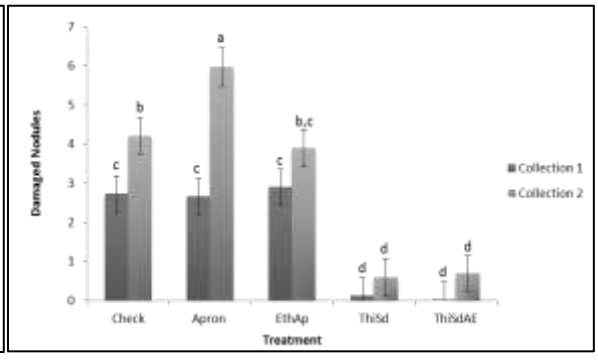

Figure 4. PLW-damaged nodules over two sampling periods in plants grown from treated seed.

A01

EFFECT OF IRRIGATION AND PLANT CANOPY ARCHITECTURE ON WHITE MOLD DEVELOPMENT IN DRY BEAN

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INTRODUCTION: Dry bean (*Phaseolus vulgaris* L.) is a profitable pulse crop in southern Alberta. White mold (WM) caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary can be a major constraint to dry bean production. Registered dry bean cultivars are either susceptible or have partial field resistance (avoidance) to WM. In favorable conditions, sclerotia present in the soil germinate and produce apothecia which release ascospores that initiate WM disease development. Soil moisture within the top 5-cm is very critical for sclerotial germination (Wu and Subbarao, 2008), and a temperature of 12-18^{o}C for several weeks is favorable for apothecia development (Hall, 1994). Plant architectural traits, such as lodging resistance and porous canopy may influence microclimate within the plant canopy, and thus avoid WM in dry bean (Miklas et al., 2013). In the semi-arid conditions of southern Alberta, dry bean is grown under irrigation which results in favorable conditions for WM development. Our objective was to evaluate the effect of irrigation and plant architecture on microclimate and WM development in dry bean genotypes.

MATERIALS AND METHODS: Studies were conducted in sclerotia-inoculated fields at Lethbridge, AB in 2015 and 2016. Three levels of irrigation (high: 1", medium: 3/4", and low: 3/5" water per week, respectively) and five genotypes with different canopy architecture (determinate bush CDC Pintium, Indeterminate bush AAC Burdett and AC Island, and indeterminate prostrate I9365-31 and Othello) were arranged in a split-plot design. I9365-31 is a black bean line with partial genetic resistance to WM (Miklas et al. 1998), AAC Burdett is a pinto bean cultivar with avoidance to WM; and the remaining three pinto bean cultivars are susceptible to WM. Dry bean plots were evaluated for WM severity and incidence, flower infection, yield and thousand seed weight (TSW). Microclimate variables, including soil moisture at 0-5 cm, soil temperature and leaf wetness was monitored using data loggers and sensors. Flower infection was assessed by plating flowers onto sclerotinia-specific media. WM severity was assessed on a 1-4 scale (Balasubramanian et al., 2014) where 1= healthy and 4= dead plant. Data were analyzed by PROC MIXED procedure of SAS 9.2.

RESULTS AND DISCUSSION: WM severity, incidence and flower infection were significantly higher in high irrigation plots compared to medium and low irrigated plots (Table 1). Significantly higher moisture within the top 5-cm of soil, leaf wetness and cooler soil temperature were maintained in high irrigation plots; all of these were conducive for WM development that persisted for several weeks (Figure 1). Highest yield and TSW were observed in plots grown under medium irrigation in both years (Table 1). Susceptible cultivars, Othello and CDC Pintium, exhibited highest WM severity, incidence and flower infection (Table 2). WM development was significantly lower in I9365-31, AAC Burdett and AC Island (Table 2). Thus, medium irrigation and the choice of cultivars could be effective tools in managing WM in Alberta. This experiment will be repeated in 2017.

Table 1. Effect of irrigation on white mould disease, yield and TSW in 2015 and 2016.

Irrigation	Year 2015					Year 2016				
	WM severity (0-4 scale)	WM incidence (%)	Flower infection (%)	Yield (Kg/ha)	TSW (g)	WM severity (0-4 scale)	WM incidence (%)	Flower infection (%)	Yield (Kg/ha)	TSW (g)
High	1.56 A	23.53 A	13.06 A	3073 B	282 C	1.54 A	22.0 A	13.93 A	4482 B	345 B
Medium	1.15 B	9.06 B	5.13 B	4683 A	332 A	1.26 B	11.2 B	10.06 B	5537 A	364 A
Low	1.14 B	8.60 B	4.80 B	3151 B	313 B	1.25 B	9.9 B	8.16 B	3904 C	346 B

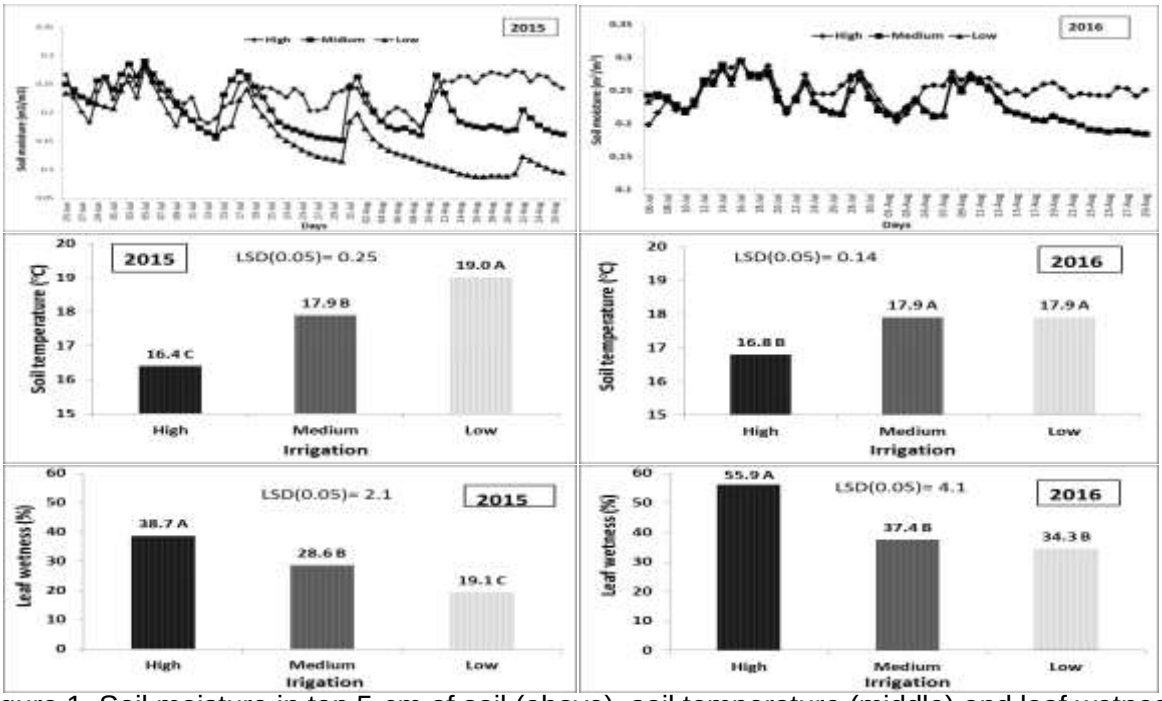

Figure 1. Soil moisture in top 5-cm of soil (above), soil temperature (middle) and leaf wetness (bottom) in 2015 and 2016 following high, medium and low irrigation treatments.

Table 2. Effect of dry bean cultivars on white mould disease yield and TSW in 2015 and 2016.

Cultivars	Year 2015					Year 2016				
	WM severity (0-4 scale)	WM incidence (%)	Flower Infection (%)	Yield (Kg/ha)	TSW (g)	WM severity (0-4 scale)	WM incidence (%)	Flower Infection (%)	Yield (Kg/ha)	TSW (g)
I-936531	1.03 B	2.66 C	1.33 C	2396 D	166 D	1.01 D	1.11 D	3.11 C	4067 C	200 C
AAC Burdett	1.13 B	7.33 BC	6.22 B	4354 A	362 A	1.20 C	7.89 C	8.67 B	4681 B	385 B
AC Island	1.19 C	10.83 B	9.44 AB	4148 AB	330 B	1.22 C	10.22 C	10.44 B	5385 A	397A
Othello	1.56 A	22.67 A	12.11 A	3846 BC	313 C	1.75 A	30.00 A	17.78 A	4827AB	379 B
CDC Pintium	1.50 A	25.11 A	9.22 AB	3416 C	374 A	1.55 B	22.67 B	13.44 AB	4909AB	399A

ACKNOWLEDGEMENT: We would like to thank Dr. Phil Mikals for providing seeds of the black bean line I9365-31, and the research technicians for assistance with the field work.

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AO2

A SUMMARY OF 15 YEARS OF RESEARCH ON THE CULTURAL AND CHEMICAL CONTROL OF ANTHRACNOSE IN DRY BEAN.

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Dry bean anthracnose (*Colletotrichum lindemuthianum*) is a serious fungal disease of dry bean, with visible symptoms on all aerial parts of the plant. Sporadic field infections have been noted in Ontario for decades, with seed yield and quality losses reaching as high as 100%. At least five new anthracnose races have been documented in Canada in the last 20 years, but the introduction of race 73 in 2001 was a game changer, as a dramatic increase in field infection occurred in Manitoba and Ontario by 2005.

In 2006, a survey of 88 commercial cultivars across 8 market classes identified a range in genetic resistance to race 73. However, there were few resistant cultivars in the navy, black and pinto market classes - the three largest commercial market classes in Canada. At the same time, plant breeders began to incorporate resistance to race 73 into the navy, black and pinto market classes, which has led to at least six new resistant cultivars entering the marketplace since 2012.

Chemical control has focused on identifying new seed treatment and foliar fungicide compounds for the control of anthracnose. Initial seed treatment studies in 2005-06 determined that Cruiser Apron Maxx Dynasty (thiamethoxam + fludioxonil + metalaxyl-m + azoxystrobin) was equal to DCT (diazinon + captan + thiophanate-methyl) for the control of anthracnose, and it was much easier to apply to seed. Recent seed treatment research has determined that several other compounds (sedaxane, ipconazole, trifloxystrobin and penflufenacil) are ineffective in controlling anthracnose. Microwave radiation was evaluated as an alternate to seed treatments for the control of anthracnose on dry bean seed. Radiation was limited to 40-50 s to minimize a reduction in seed germination. A microwave treatment resulted in a 30% reduction in disease severity in a lab assay, but no differences were found in field studies. An initial foliar fungicide study determined the best timing for fungicide application was at first flower, and that more consistent results occurred with a second application at full flower. Pyraclostrobin and azoxystrobin were found to perform similarly under moderate disease pressure, but pyraclostrobin was a superior product under severe disease pressure. More recent research in 2014 and 2015 identified several other foliar fungicides (fluazinam, fluopyram, thiophanate-methyl and picoxystrobin) that are efficacious for anthracnose, as well as other common foliar diseases such as white mold. Since the best timing for fungicide application is similar for anthracnose and white mold, growers can control the two diseases simultaneously with these products.

Cultural controls focused on anthracnose transmission between infected and uninfected plants, and the overwintering of the disease. Anthracnose can be transmitted via common materials such as denim, leather, metal and rubber. Transmission increases dramatically in a wet bean canopy, particularly for denim and leather. A natural inoculum source fell between a 1×10^5 and a 1×10^7 artificial inoculum source for disease transmission. In addition, the natural inoculum had much higher disease transmission in a wet vs dry canopy, compared to the artificial inoculum sources. To combat anthracnose transmission, eight disinfectants were evaluated in a lab study. Only three products (10% household bleach, Aqua Care FV and Dettol) were efficacious. To date, field studies have not

conducted to determine the efficacy of disinfectants or their interaction with demin, leather, metal or rubber. Two separate anthracnose overwintering studies have been conducted in Manitoba and Ontario. In 2005 and 2006, a crop rotation study using a field assay determined that plant infection occurred 20 months after the deposit of infected residue on the soil surface. In a residue overwintering study (ongoing) which uses a lab assay, plant infection occurred 18 months after the deposit of infected residue on the soil surface, but did not occur 6 months after infected residue was incorporated into the soil.

Before 2001, anthracnose was present in both seed and production fields of dry bean, but damage was sporadic. Race 73 was identified to Canada in 2001; over the next few years, it aggressively spread in seed and commercial production fields in Ontario and Manitoba. Pyraclostrobin was registered for anthracnose control in dry bean in 2002, followed by azoxystrobin in 2005. The Canadian dry bean industry made dramatic changes to their pedigreed seed multiplication beginning in 2005, by shifting the first two years of seed multiplication to dry land regions (e.g. Idaho), followed by limited (1 year) multiplication in more humid environments (e.g. Ontario). By 2006, the Ontario seed industry initiated the use of new seed treatments (Cruiser Apron Maxx Dynasty) and the timely application of foliar fungicides (pyraclostrobin and azoxystrobin). All of these actions resulted in a dramatic decrease in the incidence and severity of anthracnose in dry bean seed and production fields. From 2010 to 2013, anthracnose was identified in a few fields. Since 2014 there has been no anthracnose infection in seed and commercial production fields in MB or ON. While research into the genetic and cultural control of anthracnose is important, it must be noted that the cornerstone to the management of this disease is the maintenance of disease-free seed stocks.

AO3

OPTIMAL PLANT SPATIAL ARRANGEMENT FOR DRY BEAN (*PHASEOLUS VULGARIS)* PRODUCTION IN MANITOBA

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Manitoba accounts for a large proportion of dry bean (*Phaseolus vulgaris*) production nationally. Current production recommendations need to be revisited for modern varieties. Optimal plant spatial arrangements combine row spacing and plant stand density and have been previously shown to be a critical requirement for yield maximization through early resource capture as well as play a key role in increasing the crop's ability to tolerate biotic and abiotic stresses. Since Manitoba has a relatively short growing season early acquisition of resources is essential to maximizing plant productivity. This study aims to determine the optimal plant spatial arrangement for different varieties to maximize yield for dry bean production. Field experiments were conducted at Carman and Portage la Prairie, Manitoba in 2015 and 2016. Plant spatial arrangement was evaluated for both navy and pinto beans. For each market class two varieties were chosen with differing plant architectures and planted at row spacings of 20, 40, 60, and 80 cm. Navy beans were planted for five target population densities ranging 20- 60 plants m^{-2} and pinto beans were planted ranging five target populations of 10-50 plants m^{-2}. Initial results indicate that row spacing has a significant effect on dry bean seed yield for all varieties across locations. The narrowest row spacing of 20 cm produced the highest yields while the effect of density was inconsistent.

AO4

VOLUNTEER CANOLA IN SOYBEAN: PREVENTATIVE SEEDBANK MANAGEMENT

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Introduction

Based on seeded acreage, soybean (Glycine max (L.) Merr.) production in Manitoba has doubled in the last half decade[1]. As a result, soybean is now the third most grown crop in Manitoba, aside from canola and wheat[1]. Volunteer canola (*Brassica napus* L.), originating from large canola seed losses at harvest[2], is now among the top five most abundant weed species in western Canada[3,4]. Adventitious presence of unwanted herbicide resistance traits in pedigreed canola seedlots[5], pod drop and shatter[6], seedbank persistence[7], and seed return in subsequent crops all contribute to populations of glyphosate-resistant volunteer canola that can endure most conventional crop rotations practiced in this area[8]. Volunteer canola is a particularly problematic weed in soybean due to limited options for effective herbicide management, and an integrated approach to weed management is therefore warranted. This study focused on the evaluation of timing and implement of soil disturbance for post-harvest seedbank management to deplete volunteer canola population densities prior to soybean production.

Materials & Methods

This experiment was conducted at three sites in (near Carman, Howden & Melita) in 2013/2014 and two sites (near Carman & Pilot Mound) in 2015/2016 in southern Manitoba. Following canola harvest, and additional seedbank supplementation (7000 seeds m^{-2}), spring tooth tine harrow (1cm depth) or tandem disc (12 cm depth) were used to disturb the soil seedbank in early autumn (shortly after canola harvest; September), late autumn (one month after canola harvest; October) or spring (May), in separate treatments. A randomized complete block design with four blocks per site was used with an augmented factorial treatment structure to compare timing and implement of soil disturbance to a zero tillage control. The density of viable seeds in the autumn seedbank (upon experimental initiation) and spring seedbank (prior to spring seedling recruitment) were quantified using the soil core germination method[7], while seedling population densities in autumn and spring were evaluated using randomized quadrat counts. Absolute seedbank and seedling densities were used to derive the demographic life-stage transition rates (proportional transitions) of autumn seedling recruitment, seedling survival over winter, seed survival in the soil seedbank, and spring seedling recruitment in addition to overall population persistence from autumn to spring. Demographic rates were analysed using mixed model ANOVA in SAS 9.4 (SAS Institute, Inc., 2015), where timing and implement of soil disturbance and site were considered fixed effects while experimental block nested within site was considered random. Outliers were removed based on Lund's Test, and the square root transformation was used to adjust for normality and homoscedasticity, when necessary. To control family-wise error, Tukey's HSD ($\alpha = 0.05$) was used for multiple comparison of differences among means.

Results & Discussion

Timing of soil disturbance had a greater effect on volunteer canola population persistence than implement. Early autumn soil disturbance resulted in approximately double the rate autumn seedling recruitment and half the population persistence from autumn to spring, compared to zero tillage (Table 1). Almost all autumn recruited volunteers did not survive the winter (data not shown). Therefore, disturbing the soil in early autumn, regardless of implement, can deplete the volunteer canola seedbank via autumn recruitment and subsequent winterkill. Furthermore, spring soil disturbance stimulated spring volunteer recruitment (Table 1). Hence, low-disturbance in spring can reduce volunteer canola recruitment in-crop.

Table 1. The main effect of timing of post-harvest soil disturbance on the rates of population persistence, autumn seedling recruitment, winter seed survival and spring seedling recruitment of volunteer canola among all experimental sites.

Timing	Population persistence	Autumn seedling recruitment	Winter seed survival	Spring seedling recruitment
		%		
Zero tillage	6 ab	20 b	8 a	3 b
Early autumn	3 c	38 a	6 a	3 b
Late autumn	4 bc	23 b	6 a	3 b
Spring	6 a	23 b	9 a	11 a

Values are back-transformed square root means. Within columns, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

These results indicate that timing of soil seedbank disturbance is an effective tool that should be included in an integrated weed management strategy to decrease volunteer canola densities preceding soybean production. Interestingly, these results contradict current management recommendations based on European research conducted on winter oilseed rape[9,10,11]. Due to differences in winter-vs. summer-annual life cycles in addition to common tillage practices and climatic conditions, it is therefore suggested that recommendations for post-harvest soil disturbance of volunteer canola harvest seed losses in western Canada are updated based on these new findings.

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AO5

EFFECT OF SEED MATURITY ON PHYSICAL DORMANCY OR HARD SEEDEDNESS IN DRY BEAN

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Introduction:

Seed impermeability in legumes is often termed as 'hard-seededness' while the impermeable seeds being referred to as 'hard seeds' (Taylor, 2005). Physical dormancy results in hardseededness but its exact mechanism is unknown (Baskin and Baskin 2004; Bewley et al., 2013).Sixteen families are known to have species with physical dormancy (Baskin et al., 2000, 2006), of which the legume family (*Fabaceae*) is the largest and most important. Seed coat hardness in the legumes is an important mechanism to regulate germination. For crop plants, hard seededness /physical dormancy is not just a biological issue, but also an economic one, because this trait has a multitude of effects in germination and cooking qualities. Hardseededness is a problem in common bean, more particularly in black beans that causes 'stone seeds', which do not take up water and thus do not germinate properly nor do they hydrate during the cooking process leading to an undesirable product. The role of the seed coat in this impermeability to water is evident by the rapid germination of scarified seeds in many crops including common bean (Gurusamy et al. unpublished). A preliminary experiment with black bean varieties susceptible and resistant to hard seededness indicated that the maturity at harvest significantly affects the percent hardseededness in the susceptible variety and there is a tentative pattern of development of hard seeds over maturity. Thus understanding the developmental stage and developmental pattern of hard seededness in susceptible varieties would enable us to agronomically manipulate this trait to obtain better seed quality. It would also lay the foundation for understanding the genetics of this trait for future breeding purposes. The objectives of this study were a) to understand the effect of harvest maturity on physical dormancy (hard seed); b) to identify the developmental stage for hard seededness in black bean; c) to identify the developmental pattern of % hard seed; and d) to render better agronomic manipulation practices to reduce the hard seed in susceptible varieties.

Materials and methods:

The experiment was conducted in Saskatoon, Saskatchewan with 2 varieties, CDC Jet (susceptible) & CDC Blackcomb (resistant) in the field in 2015 & 2016 using RCBD in long plots (2.8 m x 18m) with 6 replications. Plants were harvested at 9 maturity stages: every 2 days from 28 days after flowering (DAF) to 44 DAF. All the plants were tagged in the field on the first day of flowering and harvested based on the tags for the different maturity stages and at least 60 to 100 plants were harvested per treatment per replication. To estimate the physical dormancy (hard seed), germination studies were conducted using two types of seed treatments namely "Intact seed coat" (Full seed coat) and "Scarified seed coat" as per ISTA - Roll Towel method in a controlled growth chamber in dark @ 25±1◦C with 4 x 100 seeds per replication, per treatment and per harvesting stage. Germination counts were taken after 4 days to estimate % germination (seeds imbibed with radicle emergence), % hard seed (seeds with no imbibition) and % imbibed seeds (seeds imbibed, but no germination). Imbibed seeds that are not germinated are an indication of physiological dormancy and was estimated to separate from the physical dormancy causing hard seeds. All the data were analyzed using SAS proc mixed repeated measures model.

Results and conclusions:
Data from 2015 trial on the effect of seed maturity (harvest time) vs physical dormancy (hard seededness) in CDC Jet and CDC Blackcomb suggested a significant relationship and genotypic differences ($p<0.0001$) for % hard seed among varieties, maturity at harvest stages and the interaction (variety x harvest stage). Results from the first season trial using "intact seed coat treatment" showed that the susceptible genotype CDC Jet developed hard seed of ~ 30 - 60% when harvested 32 to 42 DAF, but this was reduced to ~ 20 % if left on the plant till after 44 DAF. Results indicated that harvesting 4-5 days early leads to reduced seed quality of up to 40% in a susceptible variety. Estimation of physical dormancy following 8 months of storage showed a similar pattern, although hard seed was reduced by 20% in CDC Jet over this time. The % hard seed was less than 5 % in the resistant variety CDC Blackcomb at all maturity /harvest stages.

Following scarification of the susceptible variety CDC Jet, all seeds germinated (>98%) regardless of the maturity stage at harvest as the seed coat scarification eliminated the impermeability barrier. There was no change in the % imbibed seed in both "intact" or "scarified" seed coat treatments in both varieties, as the imbibition with no germination is likely due to physiological dormancy which cannot be rectified through seed coat scarification as seen in physical dormancy above. However the physiological dormancy rate in both the varieties were only 5 to 10% and insignificant compared to % hard seed. A similar trend for hard seed development, % hard seed vs maturity stage and statistical significance was observed in both the varieties in the 2016 field trial as well.

Thus this study forms a basis for the development of guidelines for better agronomic practices in harvesting targeted at obtaining better seed quality. The significant genotypic variation between varieties on seed hardedness suggests that the genetics of this susceptibility trait could be further dissected out for breeding efficiency.

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AO6

Application of abscisic acid (ABA) analogs for Improving pulse crop agronomy and physiology

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Spring weathers in Canadian prairies are normally cold and wet, which causes poor germination for pulse seeds. Also, the relatively short farming season contributes to nonuniform maturity of pulse crops. These problems have been affecting pulse yield and quality since pulse production was introduced to Canadian prairies. The latest research has found that ABA plays a vital role in maintaining seed dormancy and water transpiration in the plant (Finkelstein, 2013). Via non-transgenic method to manipulate endogenous ABA level in the pulse seed or plant may address and solve these issues.

This project focuses on development novel ABA analogs to reduce ABA response in pulse crops and to demonstrate their application to promote germination under low temperature, break dormancy and hasten maturation. This project consists of two major parts: chemistry activity involving design and synthesize novel ABA analogs, and applied activities which include screening and identification of promising compounds for further investigation at the seedling and adult plant stage. To date, two ABA analogs, namely ABA 1001 and 1009, have been found to have potent anti ABA activity. ABA-1001 and ABA-1009 showed germination enhancement at low temperatures for canola, and also in preliminary assays in soybean, lentil and chickpeas. ABA-1009 also showed a potential as candidate for desiccant based on lentil seedling desiccation experiments. Further research is aimed at optimizing improved ABA analog activity and in analyzing ABA and GA levels toward determining the mode of action of the ABA analogs, all geared to developing novel plant growth regulators for pulse crops.

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AP13

DOES SOYBEAN NEEDS A STARTER NITROGEN FERTILIZER IN MANITOBA?

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Summary

Biological nitrogen fixation (BNF) contributes more nitrogen (50-80 %) in pods and seeds than soil mineral N in soybean. However, this fixation by *Bradyrhizobia japonicum* bacteria does not initiate until the V2 (second fully-expanded trifoliate) or V3 (third fully-expanded trifoliate) soybean development stage (Fehr and Caviness, 1977). Therefore, during early growth stages, soybean depends on soil mineral N for the establishment of a vigorous seedling (Harper, 1974). In addition, soybean grain yield is directly related to mineral N assimilation in the first stages of reproductive growth period (R2) and to high nitrogen fixation during the R6 stage (Fabre and Planchon, 2000). Thus soil mineral N is important for early vegetative growth, establishment of N symbiosis, and ultimately grain yield of soybean.

Soybean acreage in Manitoba is expanding. Soybean acreage increased by 16.3 % in 2016 from 2015 to 1.6 million acres (Stat Canada, 2016). Low soil temperatures that commonly occur at or after soybean planting in Manitoba have the potential to delay emergence, biological nitrogen fixation, and growth of soybean (Zhang and Smith, 1994). This abiotic stress can delay or retard root hair infection along with nodule initiation, nodule development or N assimilation (Bordeleau and Prévost, 1994). Small doses of N fertilizer can prove beneficial to plant development and subsequent nodulation and N fixation, especially when initial nodulation is reduced or delayed (Mahon and Child, 1979). Studies conducted in South Dakota had shown an average 6% increase in grain yield, early biomass (V3-V4 and R1) and plant N when a starter dose of 16 kg N /ha was applied compared with no N fertilizer application in two of the three years (Osborne and Riedell, 2006). The impact of small rates of starter N on soybean grown in the Canadian Prairies has not been assessed.

A field study was initiated in 2015 with an objective to evaluate the effect of starter N fertilizer on soybean nodulation, growth, and seed yield at the University of Manitoba Research Farm, near Carman, Manitoba. Soybean (DKC 25-10 RR), inoculated with Brady rhizobium inoculant (Cell-Tech) was planted at a target population of 444, 600 plants /ha on 15-inch row-spacing. Soil at this site was an imperfectly drained Gleyed Black Chernozem (Elm Creek Series) with low soil nitrate N (44 kg /ha in top 60 cm soil layer), low soil organic matter (1.8%) and soil pH of 5.3. Treatments included six N fertilizer rates (0, 17, 34, 50, 67 and 84 kg N /ha) in a randomized complete block design with four replications. Nitrogen as urea was broadcast and incorporated into the soil before planting of soybean. Plant roots, above ground plant biomass, and soil samples were collected at R1 and R5.5 stages of crop to evaluate nodulation, soybean growth, and nitrogen uptake. Data were analysed by ANOVA using Proc Mixed procedures of SAS version 9.4 utilising a critical p value of 0.05.

Data collected from the first year of the study showed that low rates of N fertilizer application at planting did not influence soybean performance except nodule count at R1 stage. No statistical differences were observed in crop biomass, N uptake, grain yield or grain quality between N fertilizer treatments and control at Carman 2015 (Figure 1). Increasing the rate of N fertilizer significantly reduced the nodule number relative to the control ($p=0.0494$; Figure 2) but this difference did not persist at the R5.5 stage. To validate these findings, this experiment will be repeated in 2016 and 2017. A plan to examine the impact of N fertilizer application rates on soybean N fixation is ongoing.

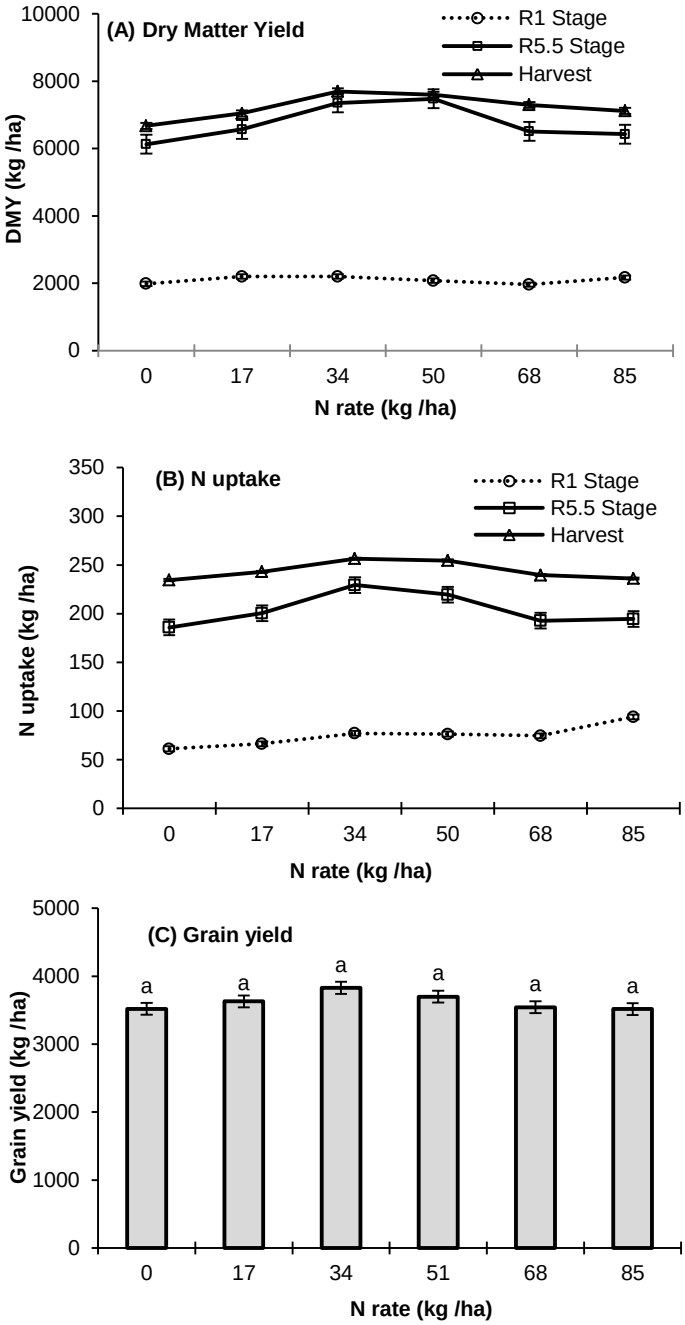

Figure 1: The effect of starter N fertilizer application rates on (A) dry matter yield, (B) plant N uptake at R1 stage, R 5.5 stage and at harvest and (C) soybean grain yield at Carman in 2015 ((Means with same letters (a,b,c) are not significantly different at the 0.05 probability level; Error bars represent the standard errors of means)

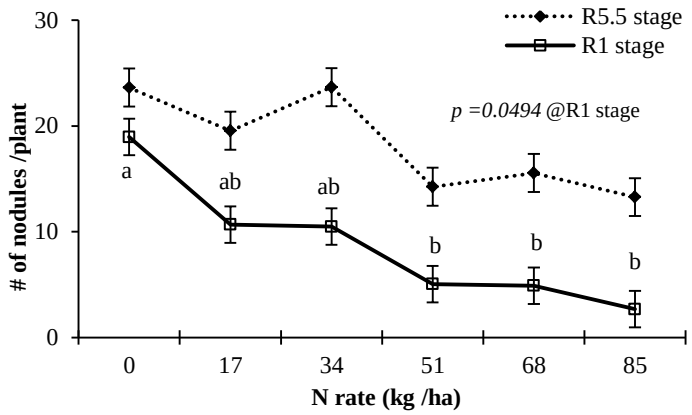

Figure 2: The effect of starter N fertilizer application rates on the number of nodules per plant at R1 and R5.5 stages of soybean growth at Carman in 2015. (Means with different letters (a,b,c) are significantly different at the 0.05 probability level; Error bars represent the standard errors of means)

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AP14

ON-FARM SOYBEAN CULTIVAR EVALUATION FOR SUITABILITY TO ORGANIC PRODUCTION IN SOUTHERN MANITOBA

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Introduction and Objective

Soybean (*Glycine max* Merr.) is not a common grain legume field crop grown on Canadian organic prairie farms, as only 119 hectares of soybeans were sown in 2014. Increasing organic soybean hectares requires technical knowledge and suitable cultivars that have been tested under organic conditions. At the moment, non-genetically modified (GM) cultivars are bred and performance tested under conventional conditions.

The objective of this research was to evaluate the performance of 12 non-GM short-season soybean cultivars on organic farms in Manitoba.

Materials and Methods

The research compared 12 non-GM short-season soybean cultivars sourced across Canada and North Dakota (Table 1), and took place at six locations (Carman, Elie, St. Pierre-Jolys, Somerset, Swan Lake, and Woodmore) in southern Manitoba between 2014 and 2015 (Figure 1). Within each treatment, a sub-plot was kept weed-free by hand to compare relative weed competitiveness of cultivars. Additional weed control included pre-emergence harrow, and inter-row cultivation at V1-V2.

Table 1. Cultivars evaluated, source, and company heat units (CHU)

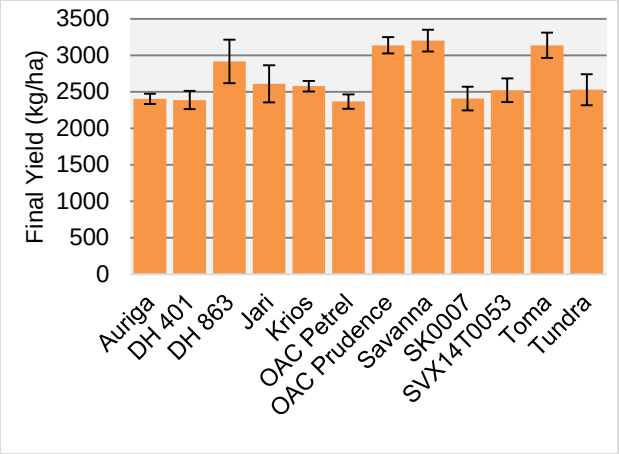
Cultivar	Source	CHU
Tundra	Semences Prograin, Quebec	2350
SK0007	SK Foods, North Dakota	2375
OAC Prudence	Robert Weins, Domain, Manitoba	2450
Toma	SemencesPrograin, Quebec	2500
DH 863	SevitaInternational, Ontario	2500
OAC Petrel	SG Ceresco, Quebec	2520
Jari	Elite Le Coop, Quebec	2550
DH 401	Sevita International, Ontario	2550
SVX14T0053	Sevita International, Ontario	2625
Auriga	Elite Le Coop, Quebec	2625
Savanna	Homestead Organics, Ontario	2650
Krios	Elite Le Coop, Quebec	2675

Results and Discussion

Yield Performance

The final performance of soybean cultivars differed depending on the site in which it was grown. For simplicity, two sites (Carman 2015, and Somerset 2015) will be used an example.

The top yielding site out of all nine site-years was Carman 2015 (Figure 1), with yields ranging from 2386 – 3200 kg ha^{-1}. At this site, yields matching or even exceeding conventional soybean yields were observed. The top producing cultivars were ‘Savanna’, ‘Toma’, and ‘OAC Prudence’. The yields at the ‘lower’-yielding site, Somerset 2015 (Figure 5), ranged from 880 – 1215 kg ha^{-1}. The top producers in Somerset 2015 were ‘Toma’, ‘SK0007’, and ‘SVX14T0053’.



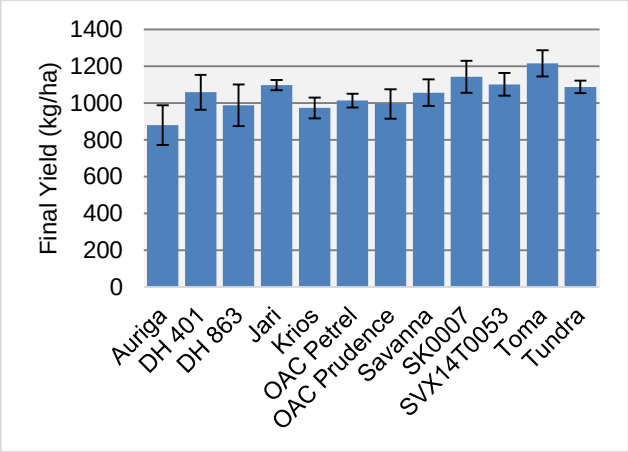
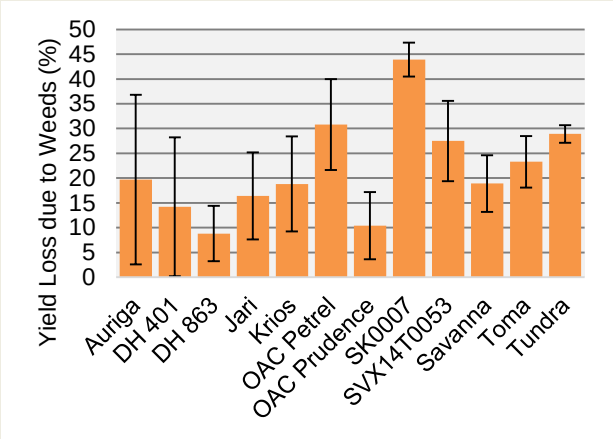


Figure 1. Final soybean yield at Carman 2015 (left), Somerset 2015 (right). Differences in letters indicate significant differences between cultivars.



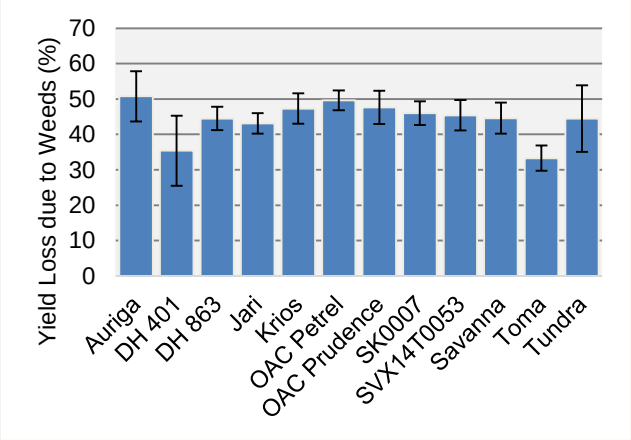


Figure 2. Percent yield loss due to weeds in Carman 2015 (left) and Somerset 2015 (right)

Interestingly, cultivar yield significantly differed from each other at Carman 2015, but not at Somerset 2015. Yield loss due to weeds was the highest at Somerset 2015, with approximately 44% lower yields in the weedy than weed-free (Figure 2). Carman 2015 saw a 20% yield reduction. These results could provide some evidence to suggest that intense weed competition stifled the potential for genetic expression. This is valuable to organic farmers in the Canadian Prairies, as cultivar choice may not be as important as a rigorous, well-timed weed control regiment isn't in place. The weeds present at Somerset 2015 are representative of the most challenging weeds organic farmers in Manitoba deal with, wild mustard (*Sinapsis arvensis*), and wild oat (*Avena fatua*).

Conclusions

High organic soybean yields are attainable in southern Manitoba, but this hinges on the ability for proper weed management and environmental conditions. Soybeans are not very competitive against cool season weeds such as wild mustard or wild oat, but may be able to compete against warm season weeds such as redroot pigweed fairly well.

Acknowledgements

The author is greatly appreciative of the financial support provided by the Natural Sciences and Engineering Research Council of Canada, Growers International Organic Sales Inc., and the Manitoba Pulse and Soybean Growers. Immense thanks to the organic farmers who collaborated and donated their land for this work.

AP15

LENTIL-BASED CROP ROTATIONS LOWER THE CARBON FOOTPRINTS

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Strategies and practices are required to increase crop productivity while reducing environmental footprints in farming. The adoption of intensified cropping systems, such as, reducing the frequency of summer fallow, including pulses in rotation, and use of higher inputs to increase biomass for soil organic carbon enhancement, has been shown to increase the system productivity compared with traditional wheat-based monoculture. However, little is known about how a wheat rotation with an annual pulse crop would perform in comparison with continuous wheat in terms of system productivity and their carbon footprints. In this study, we quantified the carbon footprints and N use efficiency of two typical cropping systems, lentil-wheat (LentW) rotation and continuous wheat (ContW), under dry, normal, and wet growing conditions.

Materials and Methods

The study was carried out near Swift Current, SK. Lentil was rotated with wheat in alternate years for 25 years, which was compared with continuous wheat. Each plot was 10 X 20 m in size. In each year, soil NO_3-N (0–0.6 m depth) and soil P (0–0.15 m depth) were measured in each plot in fall just before freeze up and these values, along with nutrient application guidelines provided by the Soil Testing Laboratory of the University of Saskatchewan, were used to determine fertilizer rates to be applied in the spring to the following crop. All inputs and outputs were recorded each year in each plot. The carbon footprints of the two systems were calculated using a Full Life Cycle analysis.

Results and Discussion

Averaged over 25 years, wheat in the lentil-wheat rotation produced a similar quantity of grain as in the continuous wheat, averaging 1860 ± 150 kg ha^{-1} yr^{-1}, but the lentil-wheat system did so with 29% less N fertilizer per year (Table 1). Fertilizer N use efficiency for wheat in the lentil-wheat system averaged 80% greater than for the continuous wheat in dry years, 97% greater in normal years, and 36% greater in wet years (Table 1).

Table 1 Wheat grain yield and its relation to fertilizer-N and N use efficiency (NUE).

Water Availability	Cropping System	Grain yield			N Fertilizer	NUE
		Mean	Min	Max	Mean	Mean
		---------- kg ha^{-1} ----------			kg ha^{-1}	kg grain kg^{-1} of N
Dry	**LentW**	1021	218	2063	22.9	73.6
	ContW	1086	201	1712	38.7	40.8
	LSD (0.05)	212	--	--	--	37.4
	***P*-value**	0.15	--	--	--	0.34
Normal	**LentW**	2180	1040	3484	34.1	126
	ContW	2054	982	3130	45.5	63.9
	LSD (0.05)	205	--	--	--	48.7
	***P*-value**	< 0.01	--	--	--	0.04
Wet	**LentW**	2389	2104	2594	37	65.2
	ContW	2248	1566	2917	48.1	47.9
	LSD (0.05)	243	--	--	--	27.6
	***P*-value**	0.03	--	--	--	0.03
Mean	**LentW**	1897	218	3484	31.5	99.1
	ContW	1822	201	3130	44.1	54.2
	LSD (0.05)	138	--	--	--	27.3
	***P*-value**	< 0.01	--	--	--	< 0.01

Over the 25 years, the soil under lentil-wheat system gained 1039 kg ha^{-1} of organic matter per year, which was 26% more than that under monoculture wheat (Fig. 1). Offsetting the carbon emissions from various inputs, the total emissions during crop production became negative, averaging -379, -634, and -580 kg CO_2 eq ha^{-1} yr^{-1}, in dry, normal, and wet years for the lentil-wheat rotation, respectively (Fig. 2). The lentil-wheat rotation had a negative carbon footprint of -0.377 kg CO_2 eq per kg of grain produced, which was substantially lower compared with wheat monoculture (-0.146) (Fig. 2).

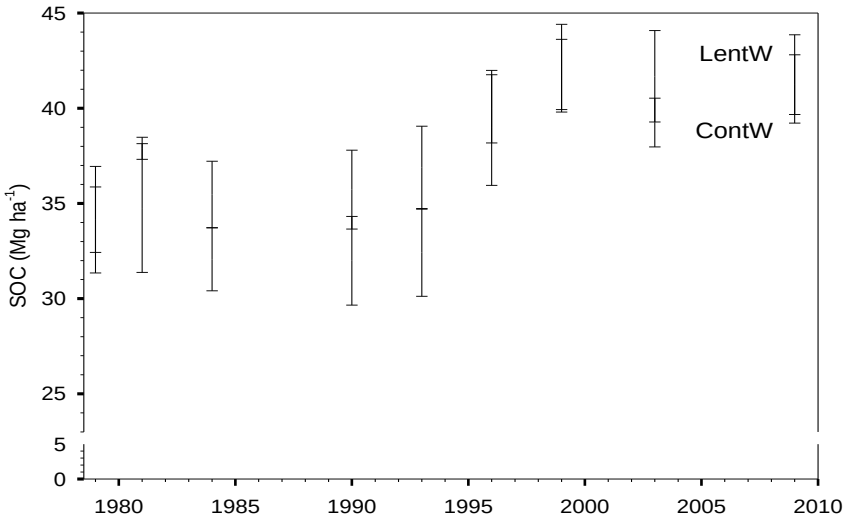

Fig. 1 Soil organic carbon in the 0–15 cm depth under LentW and ContW cropping systems.

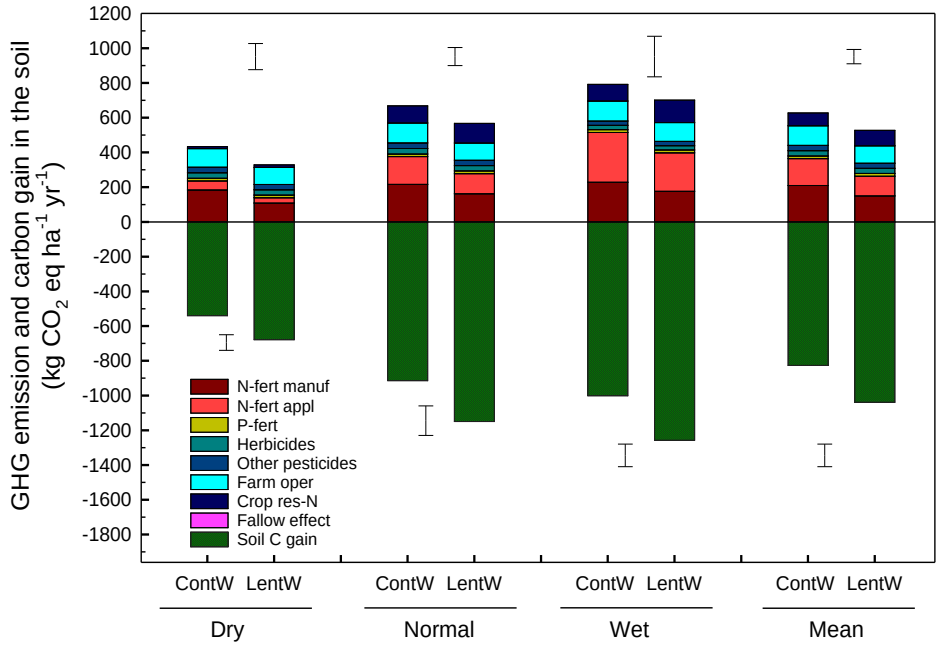

Fig. 2 Carbon emissions (top) and sequestrations (bottom) for LentW and ContW cropping systems.

Conclusions

The choice of crop rotation systems plays a key role in influencing N use efficiency and carbon footprints. Wheat grown in lentil-wheat rotation increased N use efficiency and lowered the carbon footprint significantly compared with wheat monoculture. Reduced N use and enhanced soil organic carbon over the years contributed the lowered carbon footprints in lentil-wheat rotation.

AP16

EVALUATION OF PEA (*PISUM SATIVUM*) RHIZOSPHERE BACTERIA AS BIOINOCULANTS FOR THE CONTROL OF APHANOMYCES ROOT ROT

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Aphanomyces root rot caused by *Aphanomyces euteiches* is one of the most destructive root disease of leguminous plant species, most notably field pea. *Aphanomyces root rot* is an emerging problem that could significantly affect the sustainability of field pea production in western Canada. This pathogen causes severe rotting of the root, cortex, and epicotyl that results in stunting and yellow and wilting leaves, and can result in complete loss of productivity. Currently, disease avoidance and crop rotation practices are the recommended control measures. Therefore, the search for other effective control measures is needed. One such alternative is the development of a biological control method. Since, root infection is initiated in the rhizosphere, the fundamental requirement of a biological control agent is its ability to successfully establish and survive in this zone. This research project involves laboratory and growth chamber experiments to assess rhizosphere bacteria as biocontrol agents against *A. euteiches.* An *in vitro* assay was developed to identify rhizosphere bacteria with antagonistic activity. Screening of over 150 rhizosphere bacteria identified 15 that inhibited Aphanomyces growth on lab media. Future studies will assess promising biocontrol agents as soil inoculants in pot experiments.

AP17

PHYTOPHTHORA STEM AND ROOT ROT OF SOYBEAN IN MANITOBA: ISOLATION AND RACE CHARACTERIZATION.

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Introduction

Phytophthora stem and root rot is one of the most damaging diseases of soybean, causing severe losses worldwide. The disease is mostly managed by the selection and use of race-specific or single *Rps* genes that control resistance, and quantitative or partial resistance. To date, 14 *Rps* genes have been reported. A survey conducted in Ontario, Canada during 2010 to 2012 showed that new races and more complex pathotypes have emerged since the last Canadian survey in the late 1980's (Xue et al. 2015). Different protocols and cultural media to isolate *P. sojae* from infected tissues have been reported. Most of the recommended cultural media contain expensive and toxic fungicides and antibiotics, and the protocols are challenging to follow. Manitoba's soybean industry has grown rapidly in recent years and *Phytophthora* spp. were discovered in diseased soybean roots in 2011. The objectives of this study were to find a simple approach for the isolation and characterization of *P. sojae* from infected commercial field samples and characterize the current races and virulence profiles of this pathogen in Manitoba.

Materials and Methods

Forty-four soybean fields were evaluated in 2014 for the presence of phytophthora root and stem rot symptoms. Recovery of *P. sojae* from plant samples was conducted by modifying several procedures and cultural media. Stem pieces from individual plants were surface sterilized and the central area was cut in half across the transition zone (between brown and green tissue) and placed onto modified 3-P medium. Hyphal tips of *P. sojae* that grew from infected tissue were individually transferred onto the modified 3-P medium. Single hyphal tip cultures were flooded with sterile water and one mL of water with zoospores was transferred to water agar. Four germinated zoospores from each single hyphal tip culture were transferred to the modified 3-P medium. Single zoospore cultures were then transferred to a modified pea-rye medium and DNA extraction was performed by modifying the protocol of Mahuku (2004). Species identity was confirmed by sequencing the internal transcribed spacer (ITS) region. Sequence homology was compared using BLAST analysis in the GeneBand and the Phytophthora Database.

A set of eight differentials, each carrying a single resistant *Rps* gene (1a, 1b, 1c, 1d, 1k, 3a, 6 or 7), and 'William' (*rps*) were used to test each of the 32 single-zoospore isolates of *P. sojae* for race

identification. The experiment was a completely randomized design with 5 plants per pot and 4 replicate pots for each differential line and William. Plants were inoculated using a modified hypocotyl wound technique of Dorrance et al. (2008) and Xue et al. (2015). After inoculation, pots were placed in trays containing water and moved into a mist chamber at 23^{o}C in the dark for 48 h. Pots were then removed and placed in a greenhouse with percent seedling mortality calculated at 5-10 days after inoculation.

Results and Discussion

Of the 44 soybean fields evaluated, seven fields were observed to have plants with Phytophthora root and stem rot symptoms in late August. The stem or petiole pieces with a transition zone of brown to green were the only useful tissues for isolation of *P. sojae*. Three easy ways to distinguish *P. sojae* from other fungi on the modified 3-p medium were determined. For producing mycelia for DNA extraction, the pea-rye medium was very good. Species identity was confirmed as *P. sojae* for 32 single zoospore isolates collected from commercial soybean fields. Four races of *P. sojae* were identified: race 4 was the most prevalent race followed by races 25, 28 and 3.

This study was undertaken to find a simple approach for the isolation and characterization of *P. sojae* from infected commercial field samples of soybean. Initial standardization of our methodology following published protocols exposed the difficulty of isolating *P. sojae* from field samples. The new protocol developed for *P. sojae* isolaton followed a simple approach and proved to be very reliable.

Conclusions

Information on identification of the major *Phytophthora* races causing root and stem rot in Manitoba is important in order to devise effective disease management strategies for producers. The stacking of different genes in soybean cultivars or the use of varieties with different *Rps* genes could potentially control the most prevalent races of *P. sojae* in the province. Improving disease control will ultimately result in increased competitiveness and profits by increasing yields, reducing risk and enhancing opportunities for use of soybean in crop rotations.

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Acknowledgements

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AP18

PATHOGENICITY OF *FUSARIUM OXYSPORUM* F.SP. *CONGLUTINANS* ON FIELD PEA AND DRY BEAN

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Introduction

The fungus *Fusarium oxysporum* Schlechtend.:Fr., is an economically important pathogen of many crops including canola and vegetable Brassica crops, field pea (*Pisum sativum* L.) and dry bean (*Phaseolus vulgaris* L.) worldwide. The soil-borne fungal pathogen induces vascular wilt and root rot and can cause significant yield losses on a wide range of hosts. There are host-specific strains of *F. oxysporum* that attack field pea (*F. oxysporum* f. sp. *pisi*) or dry bean (*F. oxysporum* f. sp. *phaseoli*).

In Canada, a new disease of canola known as Fusarium wilt was reported by Lange et al. (2000) and is caused by the host-specific strain *F. oxysporum* f. sp. *conglutinans* (FOC). This pathogen mainly affects canola and mustard. In canola rotations, pulse crops can provide rotational benefits. To better understand the host range of FOC and its impact on pulse crops, isolates of this pathogen were tested for pathogenicity on field pea and dry bean.

Materials and Methods

For plant material, field pea 'DS Admiral' and dry bean 'Envoy' were used for this study. Commercial growing mix (Sunshine #5; soil-less mix) was moistened and autoclaved two times for two consecutive days. For fungal material, two, three and three *F. oxysporum* strains, isolated from canola (FOC), field pea and dry bean, respectively, were used. For control treatments, only potato dextrose agar (PDA)-streptomycin media was incorporated. Seeds were surface sterilized with 2% sodium hypochlorite (NaOCl) for 5 min, rinsed with sterile water and germinated in Petri dishes on sterile moist filter paper for 3 days at room temperature in the dark. Germinated seeds were planted with 10-day old inoculum grown on PDA-streptomycin agar media using the inoculum layering technique.

The experiments were repeated twice with 4 replications of each treatment containing two plants per pot. Pots were arranged in a randomized complete block design in a greenhouse bench. Plants were grown under 24 °C/20 °C (day/night) conditions with a 16 hour light cycle and watered using a sterilized drip tube and emitter irrigation system.

After 8 weeks, root disease severity was rated on a scale of 0 (no disease) to 9 (death of plant) (Conner et al. 2011). Following rating, upper (U), lower (L) and root (R) tissue sections were plated out on pentachloronitrobenzene peptone agar (PPA) media using 3-4 sub-pieces after surface sterilization and rinsing. Following 7 days of incubation, the recovery rate (%) of each *F. oxysporum* isolate was recorded.

Results and Comments

The results from the study showed that *Fusarium oxysporum* f. sp. *conglutinans* isolates were able to successfully infect roots and stems of field pea and dry bean (Tables 1 and 2). Two isolates of FOC (#70 and #73) were recovered from root, lower and upper stems of the pea plants on PPA media. Field pea plants in which FOC isolates were isolated had root disease severity ratings with a mean of 2.85 and 2.75, respectively. On dry bean, two FOC isolates, #70 and #73, were recovered from roots and lower stems with a root disease severity rating mean of 2.55 and 1.55, respectively, based on the 0 to 9 scale. The current report provides valuable information on *F. oxysporum* f. sp. *conglutinans* based on potential alterative hosts of the pathogen, which is important when selecting non-host crops for the management of plant diseases with crop rotation.

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Table 1. Root disease severity on field pea.

Isolate	Mean[1]	Range
#70 (FOC)	2.85	2.8 – 2.9
#73 (FOC)	2.70	2.4 – 3.0
PF15-10-3 (*F. oxysporum*. f. sp. *pisi*)	2.70	1.9 – 3.5
PF15-10-14 (*F. oxysporum*. f. sp. *pisi*)	3.05	2.6 – 3.5
PF15-10-15 (*F. oxysporum*. f. sp. *pisi*)	2.80	2.5 – 3.1
Pea control	0.95	0.0 – 1.9

[1]Root disease severity was rated on a scale of 0 (no disease) to 9 (death of plant).

Table 2. Root disease severity on dry bean.

Isolate	Mean[1]	Range
#70 (FOC)	2.55	2.5 – 2.6
#73 (FOC)	1.55	1.5 – 1.6
BF11-6-10 (*F. oxysporum*. f. sp. *phaseoli*)	2.80	2.3 – 3.3
BF11-17-2 (*F. oxysporum*. f. sp. *phaseoli*)	3.55	3.3 – 3.4
BF11-17-10 (*F. oxysporum*. f. sp. *phaseoli*)	2.75	2.1 – 3.4
Bean control	1.60	1.6 – 1.6

[1]Root disease severity was rated on a scale of 0 (no disease) to 9 (death of plant).

AP19

MOLECULAR CHARACTERIZATION AND PHYLOGENY OF STEM NEMATODE, DITYLENCHUS, FROM CREEPING THISTLE OF PULSE FIELDS IN THE CANADIAN PRAIRIES

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The stem and seed nematode of creeping thistle (*Cirsium arvense*), *Ditylenchus weischeri*, was first described in 2011 in Russia based on morphological and molecular (ITS-RFLP, *hsp90* sequence) differences of the nematode from *D. dipsaci*. More recently, we reported creeping thistle in commercial fields from the provinces of Saskatchewan and Manitoba parasitized by *D. weischeri.* Here we present a detailed phylogenetic position of the nematode in relation to other *Ditylenchus* species based on molecular analyses. *Ditylenchus weischeri* from creeping thistle plants and seeds contaminating yellow pea grain samples from Canada and creeping thistle from Russia were examined. Garlic infested with *D. dipsaci* from Quebec and sequence data of other species of *Ditylenchus* retrieved from the Genbank database was also used. The Canadian *D. weischeri,* showed minor differences in morphology to the holotype type of this species from Russia. Sequences of the Internal Transcribed Spacer (ITS) region, D2-D3 expansion region of the 28S gene and heat shock gene (*hsp90*) were used to construct individual dendograms of relatedness of *Ditylenchus* species. For each of the three genes examined, *D. weischeri* grouped separately from other *Ditylenchus* species. These results provide multiple lines of evidence that *D. weischeri* is not only molecularly distinct from *D. dipsaci* but also other species of *Ditylenchus*.

AP20

DUPLEX CONVENTIONAL PCR AND REAL TIME PCR MELTING CURVE ANALYSIS OF ONTARIO POPULATIONS OF THE SOYBEAN CYST NEMATODE

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The Soybean Cyst Nematode (SCN), *Heterodera glycines*, is an import pest of soybean limiting yields in Ontario and other soybean production areas worldwide. In this study, we evaluated the suitability of two published conventional PCR, ITS and SCAR derived species specific primers sets and three new mitochondrial DNA primer sets for the CoxII, CoxIII, ND4 genes for identification of *H. glycines* from commercial fields in Ontario. The assays were done as duplex PCR with universal primer set D3A/D3B or D2A/D3B. *Heterodera glycines* from 20 commercial fields from Ontario for a total of individuals examined ranging from 122 to 156 for each of the genes. As well, samples of *H. glycines* from two states in the USA and negative controls of two other species of *Heterodera* (*H. schachtii*, *H. carotae*) and two plant parasitic nematode species for each of the genera *Ditylenchus* and *Meloidogyne* were also examined. The SCAR, CoxII and CoxIII primer sets consistently were positive for SCN individuals from Ontario and from the USA, and negative for non SCN species. The ITS and ND4 primer sets were negative for 16% and 40% of SCN individuals examined from Ontario, respectively. The results show the published SCAR primer set as well as two new mitochondrial gene based sets, CoxII and CoxIII, can identify SCN individuals from Ontario using conventional PCR. Research continues to examine the primer sets under real time assays for quantification of SCN in soil from Ontario.

AP21

LYGUS BUG FEEDING INJURY TO NAVY BEANS

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Introduction

Lygus bugs are plant bugs that are significant pests of many crops in Manitoba. Adult females lay eggs in the stems of plants and the nymphs that hatch go through five instars before their final moult to the adult stage. Adults and nymphs injure crops by piercing plant tissues and sucking the fluid contents. For the last two decades there has been concern about the effect of lygus bugs on field beans. In 2002, spraying of field beans for plant bugs was widespread in Manitoba, but there are no records for that year of the effect on field beans of the bugs or of their control. It has been reported that 5–20% of field bean seeds can be damaged by *Lygus* bugs, and, in Manitoba, seed blemishes are often diagnosed as *Lygus* damage when they are not. Characterizing the effects of *Lygus* bug feeding on field beans will help growers in early detection of *L*ygus bugs in the field, and assist graders in distinguishing lygus feeding from other sources of seed damage. As the major species of *Lygus* bug in Manitoba is *Lygus lineolaris*, our objective in the laboratory study reported here was to characterize the injury caused by *L. lineolaris* nymphs and adults feeding on three growth stages of navy beans.

Methods

Single reproductive structures (racemes) of potted navy bean plants were caged at either the R2–R3 (flowering to early pod set), R4–R5 (mid pod to early seed fill) or R6–R7 (mid seed fill to seed maturity) growth stage. For each growth stage, there were six replicates in which pre-reproductive adult *L. lineolaris* were introduced into the cage, six replicates where 5th instar *L. lineolaris* nymphs were introduced, and six control replicates with no insects. At the R2–R3 stage, there was one insect per cage; there were three insects per cage at R4–R5 and five insects per cage at R6–R7. After 5 days of feeding, the insects and cages were removed. The short-term effect of injury was characterized by detaching the raceme and examining it under the light microscope. Other racemes were grown to the time of harvest, and the effect of the earlier feeding on harvested seeds was characterized microscopically.

Results and Discussion

The type of injury differed with the growth stage of the plants, but within each growth stage, the type of injury produced by adults and by nymphs was the same. Regardless of the plant growth stage, brown discoloured areas were found in injured tissues. Part of the plant's response to injury involves oxidation of phenols by phenoloxidases, which results in brown pigmentation of injured tissues.

The most frequent result of feeding injury at the R2–R3 stage was pod abortion. Pod abortion occurs in healthy plants at this stage, and aborted pods caused by lygus bug feeding can be distinguished by brown-pigmented spots of about 1 mm diameter, centred on *Lygus* feeding sites, (Fig. 1a) and by interior necrotic lesions. Feeding injury also caused localized swellings of the pods; these were the result of interior cells swelling because feeding disrupts the plant's hormonal system. The interior cellular swelling sometimes strained the epidermal tissue so that it split. Split lesions occurred on pods and on the peduncle, those on the peduncle were mostly close to the nodes and often resulted in breakage of the stem.

At the R4–R5 stage, external indistinct brownish areas were found mainly near the ventral suture of pods. Near this suture, stylet entry points were seen penetrating to the placental region of the seed pod, and this was frequently associated with necrosis of the funiculus (Fig. 1b). Developing seeds receive minerals and photosynthates through the placenta and funiculus, and when these are

damaged the shortage of resources results in abortion and collapse of fertilized ovules. Shrivelling of seeds was seen at harvest time when feeding had occurred at R4–R5. Direct injury to seed, causing surface pitting of seed at harvest time, was an infrequent result of feeding at R4–R5.

At the R6–R7 stage, direct seed injury was more visible because, in the more mature seeds, there was greater pigmentation of the testa bordering the injury site (Fig. 1c). The injury was not localized on any particular part of the seed, and more than one injury on a seed could occur. At feeding sites, the testa was perforated and there was a cavity in the underlying tissues of the cotyledon. In most cases, it appears that stylets were inserted through the pod wall and the feeding site on the seed was close to the pod penetration point. When seeds injured during the R6–R7 growth stage were examined at harvest, lesions were crater-like pits and frequently had surrounding concentric raised ridges of brown tissue. In these seed pits, the central pit is likely to be the result of physical and enzymatic destruction of the cells of the testa and underlying cotyledon, with the surrounding pigmentation arising from the plant's phenol-phenoloxidase wound response. The occurrence of blemished seeds in a seed sample for grading would reduce the grade and also could make beans unsuitable for canning.

In field conditions in Manitoba, adults of lygus bugs are more abundant at the R2–R3 stage, and therefore pod abortions could result from their feeding. At the R4–R5 stage, later stage nymphs are dominant and losses due to seed shrivelling could occur. In Manitoba, adults and later stage nymphs migrate from adjacent harvested canola at the R6–R7 stage of field beans, resulting in high numbers of lygus bugs in the bean crop. Quality loss due to seed pitting in the R6-R7 stage could occur. Therefore how growth stage relates to the seasonal pattern of occurrence of *L. lineolaris* in commercially crops must be considered in the management of this pest.

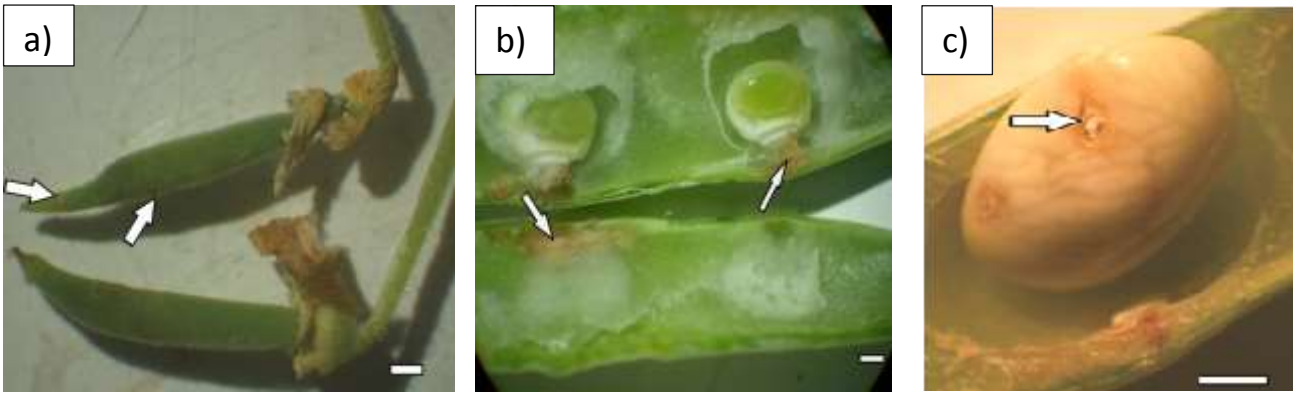

Fig. 1 Predominant injuries to navy beans at different growth stages: (a) aborted pods with brown feeding sites at R2-3, (b) injury to the placenta and funiculus at R4-5, and (c) seed pitting at R6-7. Scale bars = 1 mm.

Acknowledgements
The Manitoba Pulse Growers Association, Manitoba's Agri-Food Research and Development Initiative and the University of Manitoba for the funding. Our thanks are due to Bruce Brolley and Dennis Lange from Manitoba Agriculture for all their help with this project. We thank Dr. Parthiba Balasubramanian and Dr. Anfo Hou from Agriculture and Agri-Food Canada for providing assistance with bean growing and processing.

AP22

EVALUATING THE CRITICAL WEED FREE PERIOD OF *GLYCINE MAX* (L.) MERR. BIOMASS GROWN IN NARROW VS. WIDE ROW PRODUCTION IN NORTHERN CLIMATES

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Manitoba is located at the northern fringe of the soybean (*Glycine max* (L.) Merr.) growing area in North America. Soybeans have recently become the third most important crop based on planted acreage in Manitoba, behind wheat and canola[1]. Soybeans are a nitrogen fixing legume and as such are an excellent rotational crop for Manitoba producers who are limited in the number of well-adapted legume species that they can grow effectively. Soybeans are a C3 plant that behaves much more like a C4 plant and therefore, are better suited to warmer areas with longer growing seasons. The development of short season soybean has enabled producers in Manitoba and eastern Saskatchewan to adopt the crop for primary production. Soybeans also appear to be more tolerant to wet soil conditions than other crops, likely contributing to their recent increase in popularity. As soybean production is relatively new to the prairie region, many of the recommendations to produce soybean are being adopted from more southern areas, but these need to be tested and optimized for our climate and soybean varieties.

In the USA and Ontario, soybean production has contributed to the selection of a number of weed biotypes resistant to glyphosate. Glyphosate resistant weed biotypes of giant ragweed (*Ambrosia trifida* L.) and Canada fleabane (*Conyza canadensis* L.) have progressively moved northward into Minnesota and North Dakota from the intensive corn-soybean rotations of the US mid-western states[2]. Glyphosate-resistant Kochia (*Kochia scoparia* L.) has recently been confirmed in Manitoba and Saskatchewan[3]. With increasing use of herbicides, particularly glyphosate, in our crop production systems, more weeds are being selected for herbicide resistance each year. As part of a responsible, integrated weed management (IWM) strategy, soybean production in northern climates must adopt good agronomic practices to reduce the selection pressure for herbicide resistant weeds.

An IWM strategy uses biological, chemical, cultural, and mechanical methods to improve crop competition against weeds[4]. All methods used in an IWM must be based on an understanding of key concepts such as the critical period of weed control and relative timing of weed emergence to the crop[5]. The critical period of weed control consists of two parts: the critical time of weed removal (CTWR) and the critical weed free period (CWFP)[6], [7]. The CTWR describes the latest time weeds must be removed to limit yield losses from late emerging weeds to 5% of yield. The CWFP describes the develoipmental stages during which a specific crop must be kept weed free to limit yield losses including those from early emerging weeds. Integrated weed management tools can be used to shorten the CWFP and CTWR, although which integrated weed management approaches work best in our region to achieve this remains unknown.

Experiments for this research were located at Carman, Kelburn, and Whitemouth, MB to represent various soil types, environmental conditions, and weed communities. Soybeans were grown in narrow (19 cm) and wide (76 cm) row spacing as part of a split-plot, randomized complete block experimental design. At both row spacings, variety (Dekalb 23-60, Monsanto Canada Inc.) and seeding rate (444 600 seeds/ha) were constant. A mixture of glyphosate (444 g a.e./ha) and bentazon (538 g a.i./ha) was used to remove weeds from sub-plots until soybean's reached the VE, VC, V1, V3, V4, V5, R1, and R2 development stages[8]. At the R5 to R7 soybean development stage, crop and weed shoots were collected and dried for biomass analysis using standard procedures. Soybean shoot biomass, expressed as percentage of weed-free soybean shoot biomass, was modelled with the NLIN Mixed procedure of SAS 9.4 using the Gompertz function:

$y(t) = ae^{-be^{-kt}}$. Weed shoot biomass was analysed with the MIXED procedure and significant differences among treatment means were determined using Fischer's protected LSD (P<0.05).

Soybean biomass, in response to different durations of weed competition with soybeans, conformed to the Gompertz equation at all locations and row spacing treatments. A significant difference (P<0.05) in the 'k' model parameter between narrow and wide row soybean production was observed at the Carman location only. A lack of a reduction in soybean biomass in narrow row spacing at Carman, even in the shortest duration weed-free treatment, contributed to the significant differences in the modelled parameter estimates of the Gompertz function. At the remaining locations, soybean biomass response in narrow and wide row production did not differ. The k parameter of the Gompertz function defines the slope of the curve. Based on these preliminary results for crop biomass in 2016, the CWFP in soybean appears to have ranged from about the V1 to R2 stages.

Apparent differences among locations of the CWFP for soybean biomass production were caused by differences in weed recruitment density and periodicity. Total weed biomass of the weedy control was greatest at Carman followed by Whitemouth (P=0.0108) and Kelburn (P=0.0387). Weed biomass accumulation in the various weed free period treatments suggests different weed emergence periodicity among the locations. This is not surprising as the dominant weeds were not the same among the locations. At Carman, the weed community was dominated by *Amaranthus retroflexus* L., *Polygonum convolvulus* L., and *Setaria pumila* (Poir.) Roem. & Schult. The weed community at Whitemouth was dominated by *Avena fatua* L. and *A. retroflexus* while at Kelburn it was dominated by *S. pumila* and *Polygonum persicaria* L.

Differences among locations of the CWFP for soybean biomass production were also likely caused by differences in environmental conditions. Soil conditions at Carman were cool and dry at seeding (May 11th, 2016), with the first substantial rainfall occurring on May 23rd, 2016 and weeds emerged much later when soybeans were at the V1 developmental stage. Soybeans were planted on May 24th and 25th at Whitemouth and Kelburn, respectively. The soil had adequate moisture and temperature for seeding soybeans and weeds were actively growing at both locations. The Whitemouth location experienced heavy rainfall events in early, mid and late June. These were so severe that they likely killed the most recently emerged flushes of weed seedlings between the VC and V2 soybean development stages. Despite this, a CWFP was still observed at this location.

In conclusion, based on these preliminary shoot biomass results, a wide range in CWFP appears to exist among these locations and row spacing treatments. Differences in weed spectrum and densities as well as environmental conditions contributed to this. Nevertheless, these preliminary results suggest that the CWFP appears to be longer in wide-row soybean production systems than in narrow row production systems. Additional site years and determination of the CWFP using appropriate soybean yield data will be required to confirm these initial observations.

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AP23

COMMON BACTERIAL BLIGHT LEAF RESISTANCE IN DRY BEAN BREEDING LINES

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Common bacterial blight (CBB) of dry bean caused by *Xanthomonas axonopodi*s pv. *phaseoli* is the most economically important bacterial disease worldwide. Infection causes necrotic lesions, sometimes leading to severe defoliation. The decrease in photosynthetic area can result in up to 50% yield loss. Host resistance is the most effective and efficient control method for CBB and is controlled by both major and minor quantitative trait loci (QTL). Over 400 breeding lines from the NDSU dry bean breeding program belonging to several market classes and at various breeding stages underwent genotypic and phenotypic analysis for CBB. Genotypic analysis was performed using two dominant SCAR markers associated with major QTLs SAP6 and SU91. Preliminary genotypic results indicate 60% of the breeding lines contain SAP6, 15% contain SU91, and 10% contain both SAP6 and SU91. Preliminary phenotypic results indicate 14% of the breeding lines are resistant to CBB, 48% exhibit a moderate reaction and 38% are susceptible. Eighty percent of the resistant breeding lines contain either SAP6 or SU91. Breeding lines displaying a resistant phenotypic reaction and lacking these markers will be designated for further evaluation for the presence of other genetic regions associated with resistance to CBB.

AP24

CLIMATIC REQUIREMENTS AND VIRULENCE OF ASCOCHYTA PISI COMPARED TO PEYRONELLAEA PINODES

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Peyronellaea pinodes is generally considered the dominant and most destructive species among ascochyta blight causing pathogens, whereas *Ascochyta pisi* and *Phoma medicaginis* var*. pinodella* are considered of minor importance. Field observations and seed testing over the last 10 years has shown that for reasons unknown *A. pisi* has become more common than *P. pinodes* in the southern and south-western parts of the Canadian province of Saskatchewan. Conidial germination studies were conducted on glass slides incubated at temperatures ranging from 10 to 30°C for periods of 0 to 12 h and revealed that both pathogens had highest germination at 20 to 25°C and 10 to 12 h incubation. The same temperatures and 10 to 12 h of leaf wetness also resulted in the highest disease severity on plants of pea cultivar Cooper. *Peyronellaea pinodes* had higher conidial germination and induced higher disease severity compared to *A. pisi*, confirming that it is the more virulent pathogen. Results indicate that the prevalence of *A. pisi* in some areas of Saskatchewan is not due to differences in temperature and the length of the wetness period. Other factors, such as a higher resilience to interrupted wetness periods or differences in the relative importance of conidia versus ascospores in both species, may provide *A. pisi* with a comparative advantage in southern and south-western parts of Saskatchewan.

AP25

SURVEY SAYS: NO SOYBEAN CYST NEMATODE, HETERODERA GLYCINES ICHINOHE, IN MANITOBA

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This report details results of a survey of commercial soybean (*Glycine max*) fields for *Heterodera glycines* Ichinohe, 1952, (Soybean Cyst Nematode; SCN), conducted in 2014-2015 in the Province of Manitoba, Canada. *Heterodera glycines* is recognized as the major pest of soybean worldwide. With respect to soybean cultivation for Manitoba farmers, early detection and precise identification is of significant importance. Soybean cyst nematode has rapidly moved northward in the mid US states. It is now present in some of the counties of North Dakota and Minnesota bordering Manitoba. It is only time until it is Manitoba, if not already. Recently, the Canadian Food Inspection Agency has declassified SCN as a regulated pest in Canada. This means to farmers that surveys for the nematode are no longer done by the agency. In the current study, twenty eight fields were sampled for a total of 205 soil samples analyzed for the presence of SCN. Nematode cysts were recovered from 32 soil samples. The samples yielded one to a few cysts each, with the majority being empty and broken. Further, most cysts were round and not lemon-shaped, the later a possible indicator of SCN. Further, most cyst cone tops were circumfenestrate rather than bifenestrate, the later possibly indicating SCN. Only six samples yielded DNA suitable for PCR analysis and these were all negative for SCN. With the current and past survey conducted by the Soil Ecology Laboratory, a total of 76 commercial soybean fields in Manitoba have been sampled and are negative for the presence of SCN. Most fields in Manitoba have a history of three or less crops of soybean. That soybean acreage has recently surpassed 1,300,000 acres/year in Manitoba, it may still be a few more years until SCN populations are detected. Further, because SCN is near the North Dakota and Minnesota border with Manitoba, it is recommended surveys be conducted every two to three years.

AP26

SOYBEAN PLANTING DATES BASED ON SOIL TEMPERATURE IN MANITOBA

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Introduction

Soybean [Glycine max (L) Merr.] production has increased dramatically in Manitoba over the past decade with 1.63 million acres planted in 2016 (Stat Can, 2016). Due to this expansion, there are many new soybean growers in the province faced with the decision of when to plant soybeans. It is currently recommended in Manitoba to plant soybeans when the soil temperature at seed depth is at least 10°C on the day of planting. However, current information on the effects of soil temperature at planting is limited.

Objective

The goals of the study were to 1) determine if soil temperature at planting was an influential factor on soybean yield, emergence, and physiological maturity, and 2) identify the soil temperature that produced the highest soybean yield.

Materials & Methods

The two-year field study consisted of early (DK 23-10RY) and late-maturing (DK 25-10RY) soybean varieties seeded on six different planting dates. Planting dates were determined by the target soil temperatures of 6, 8, 10, 12, 14 and 16°C. An operational definition of soil temperature was established for this study due to the diurnal fluctuation of temperature. Soil temperature was defined as the temperature at a 5 cm depth at 10:00 AM for two consecutive days, in which seeding took place on the second day. The time of 10:00 AM was chosen to serve as a “representative” time of day for soil temperature that would allow enough time to seed on the same day.

Results

Yields

Soybean yield was the main factor considered in this study. No differences between cultivars were found for soybean yield. Reported results are averaged over the two cultivars (Figure 1). A significant relationship between soil temperature at planting and soybean yield was found only at Carman in 2015 (Figure 1B). At Carman in 2015, yields followed a quadratic model and maximum soybean yield was reached at 9°C at 10:00 AM. Beyond 9°C, soybean yields declined with further increases in soil temperature (Figure 1B). However, yield reduction in response to warming soil temperature is the opposite of what we might expect. As Carman 2015 was the only site year with planting delayed into June, the decline in yield was likely influenced by later planting rather than warming soil. Overall, yields were greater at Carman in 2014 and 2015 when soybeans were planted into cooler soil temperatures, or on earlier calendar dates (Figure 1). Although soybean yield was represented as a response to soil temperature at planting for both Carman 2014 and 2015, it is more likely that yield differences occurred due to calendar date rather than soil temperature at planting (Figure 1B).

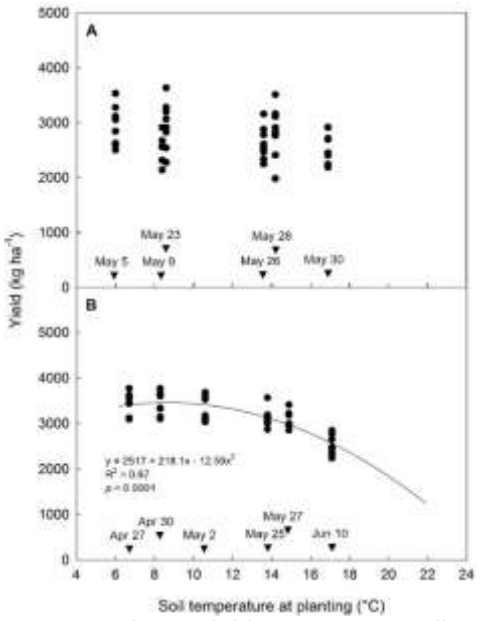

Figure 1. Soybean yield response to soil temperature at planting at **(A)** Carman, MB in 2014, **(B)** Carman, MB in 2015.

Emergence

Soybean emergence variables including days to 50% emergence, plant population at 100% emergence, and percentage seedling mortality were assessed to determine the effect of soil temperature at planting on soybean plant establishment. Again, no differences between cultivars were found for soybean emergence.

Days to 50% soybean emergence from combined 2015 site years clustered into “cool” (6 to 12°C) and “warm” (14 to 22°C) soil temperatures. Cool temperatures generally caused delayed soybean emergence, and warm soil temperatures resulted in rapid emergence (Figure 3). A significant linear relationship was found between days to 50% emergence and soil temperature at planting for only cool soil temperatures, where days to emergence decreased with increasing soil temperature at planting (Figure 3). Contrastingly, days to 50% emergence were unresponsive to warm soil temperatures (Figure 3). This result suggests that soil temperatures of at least 14°C at planting are ideal for soybean emergence.

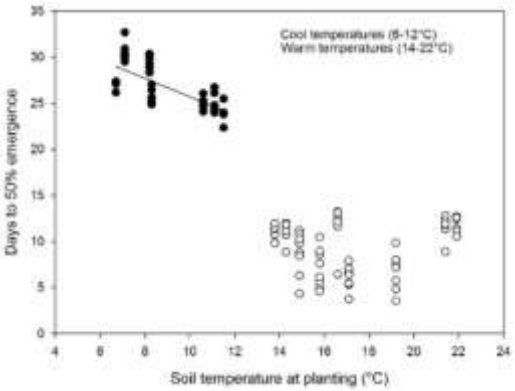

Figure 3. The relationship between days to 50% soybean emergence and soil temperature at planting for combined 2015 site years.

A significant positive relationship between soybean plant stand at 100% emergence and soil temperature at planting occurred only at Morden in 2015. At this site, established spring plant populations increased with increasing soil temperature at planting (Figure 4). A significant negative linear relationship was found between soybean seedling mortality and soil temperature at planting at Morden in 2015 (Figure 5). High soybean seedling mortality (Figure 5) coincided with low plant stands at 100% emergence (Figure 3B), suggesting that seedling mortality was responsible for low soybean plant stands at Morden in 2015.

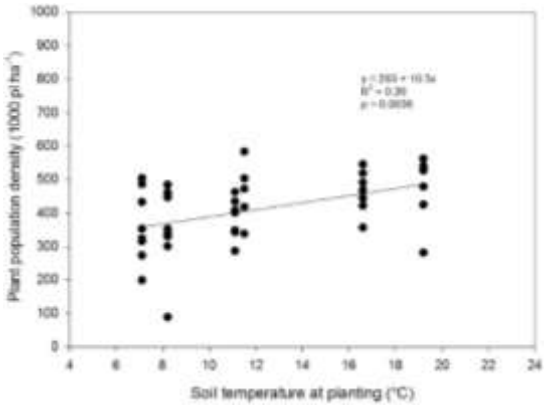

Figure 4. The relationship between maximum soybean plant population at 100% emergence and soil temperature at planting at **(A)** Carman, MB and **(B)** Morden, MB in 2015.

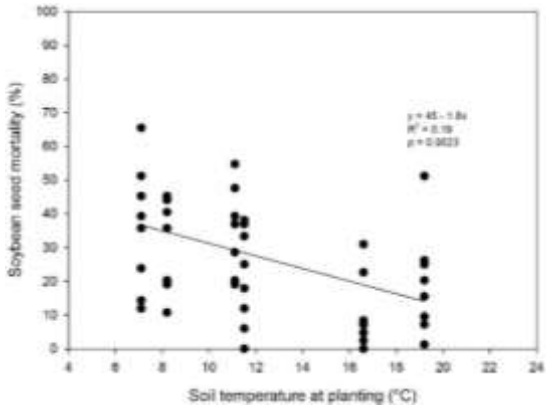

Figure 5. The relationship between percentage soybean seedling mortality and soil temperature at planting at Morden in 2015.

Frost Effect

Late spring frost events occurred at both Carman and Morden, MB on May 30, 2015; however, Carman experienced a milder frost event compared to Morden. Air temperatures ranged from -0.4 and -0.7°C over two hours at Carman, whereas temperatures ranged from -0.5 to -1.6°C for three hours at Morden. More extensive seedling damage was observed at Morden due to frost.

Conclusions

Results from this two-year study suggest that low soil temperatures at planting did not penalize yield. Calendar date and spring frost had a greater influence on soybean yield and emergence, respectively, compared to low soil temperatures at planting. Therefore, it is recommended that growers should consider calendar date, seedbed conditions, the weather forecast following seeding, tolerance to loss from spring or fall frost, and timeline to complete seeding and harvest, when determining soybean planting dates.

AP27

EFFICACY OF IN-FURROW FUNGICIDES FOR MANAGEMENT OF FUSARIUM ROOT ROT IN FIELD PEA

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Fusarium root rot often leads to substantial yield losses under the high disease pressure conditions common in North Dakota, USA. Current management tactics, including the use of seed treatment fungicides and crop rotation, do not effectively reduce symptoms caused by *Fusarium* spp. in field pea. The efficacy of in-furrow fungicide applications at planting was evaluated to manage Fusarium root rot of field pea. Field plots were not inoculated, or inoculated with *Fusarium avenaceum* or *F. solani* infested grain placed in-furrow with the seed at planting. QoI (FRAC 11), SDHI (FRAC 7), and DMI (FRAC 3) fungicides applied in-furrow were compared to non-treated plots and a commercial mefenoxam/fludioxonil (Apron MAXX RTA 2.8 g ai/45.35 kg) seed treatment. Inoculation significantly increased root rot severity in non-treated controls in all six trials. Although the performance of individual fungicides varied across site-years, the triazole fungicide prothioconazole (Proline 200 g ai/ha) always significantly reduced root rot severity in plots inoculated with each *Fusarium* species compared to the inoculated control. Prothioconazole also significantly reduced disease severity compared to the seed treatment in five of six trials. Preliminary results indicate that in-furrow fungicide applications may be an effective method of managing Fusarium root rot in field pea.

AP28

ANTHRACNOSE RACES IN MANITOBA AND ONTARIO FROM 2005 TO 2015 AND THEIR REACTIONS ON ONTARIO DRY BEAN CULTIVARS

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Anthracnose, caused by *Colletotrichum lindemuthianum*, is considered to be one of the most destructive diseases of dry bean (*Phaseolus vulgaris*) in the world. Severe early infection can result in extensive defoliation and yield losses that can be close to 100%. In Canada, a number of different races have been identified requiring major breeding efforts to introduce new cultivars with effective resistance. Between 2005 and 2015, commercial fields of dry beans in Manitoba and southern Ontario were surveyed to determine the frequency of occurrence of different races of the anthracnose fungus. Throughout the study, race 73 was most prevalent in Manitoba and Ontario. However, four anthracnose races that have not previously been reported in Canada were identified. These new races and several previously identified anthracnose races were used to screen 50 dry bean cultivars from Ontario for seedling resistance. The results of the cultivar screening and information on the virulence patterns of the new races will be presented.

AP29

Polyamine-induced changes in the expression of antioxidative genes in pinto bean seedlings under excess soil moisture

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Excess soil moisture is an abiotic stress factor that impacts the growth and developmental of plants at all stages as it triggers the production and accumulation of reactive oxygen species (ROS) that can cause oxidative damage, and seedlings in particular are vulnerable to such oxidative stress-induced damage. Extensive oxidative damage that occurs during germination or at early seedling stage can affect plant performance and productivity at subsequent stages. Antioxidative enzymes such as ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) can regulate and limit ROS production and accumulation and are considered as an important part of plants' antioxidative defense system. Polyamines such as spermidine (Spd) and spermine (Spm) have been reported to enhance the activity of antioxidative enzymes, especially under stress conditions. To gain insights into the roles of polyamines in inducing the activities of antioxidative enzymes in pinto bean seedlings exposed to excess soil moisture conditions, this study examined the expression patterns of genes encoding antioxidative enzymes in response to seed treatment with spermine and spermidine Under excess moisture conditions, *CAT* expression was induced by treatments with Spd and Spm in both cotyledon and embryo axis tissues. Similarly, expression of *APX* was activated by treatment with both polyamines but only in the cotyledon tissues. Treatment with Spd, but not with Spm, led to increased expression of one *SOD* gene, *Fe-SOD,* in the embryo axis of seedlings exposed to excess soil moisture conditions as compared to that observed in tissues derived from control (untreated) seedlings.

AP30

EVALUATION OF NATIVE BACTERIA ASSOCIATED WITH SOYBEANS FOR THE INTEGRATED CONTROL OF ROOT ROT (*PHYTOPHTHORA SOJAE)*

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Abstract
A total of 100 bacteria, isolated from rhizospheric soil, were assayed for antifungal activities against *Phytophthora sojae*.race 4.
Results indicated that 4 isolates (S1, S9, S10 and S11) were able to inhibit the hyphal growth of *P. sojae in vitro*. These isolates, selected on the basis of their strongest antagonistic activity against *P. sojae* race 4 *in vitro* were evaluated for their beneficial effects on soybeans plants in the greenhouse. Results showed that application of S11 as seed coating reduced the disease severity and enhanced plant growth, resulting in a greater protection against the infection. Additionally, a significant positive correlation was recorded between the *in vitro* and *in planta* effect of this bacteria, suggesting both a direct and indirect effect in controlling the disease.

Keywords: *Phytophthora sojae*, soybeans, biological control agents, *in vitro* inhibition, disease evaluation.

Introduction
Root rot is a major disease of soybeans that can cause significant yield reductions. The common management strategies, including seed treatment with fungicides, generally fail to provide adequate control of the disease. Alternative treatments are needed to ensure the efficacy of the limited number of fungicides available to control root rots mainly caused by *Phytophthora sojae*. Plant growth promoting rhizobacteria (PGPR) may constitute a good potential. The interaction between *P. sojae* and soybean plant in presence of PGPR was investigated under greenhouse conditions.
The objective of this study was to (i) isolate bacteria from the soybean rhizosphere (ii) and assess their ability to reduce the severity of the disease under greenhouse condition.

Material and methods
Soil samples were collected from different locations in Southern Manitoba, Canada. Soil samples were suspended in sterile distilled water and the suspension was used for isolation of rhizospheric bacteria. All isolated bacteria were tested for their inhibitory capacity against *P. sojae* race 4.
Biocontrol test were conducted with the commercial cultivar TH 32004R2Y susceptible to *P. sojae* race 4. Seeds were immersed in either sterile water (negative control) or in a standardised suspension of bacteria isolates (10^7CFU ml−1in sterile water) then planted and maintained in the greenhouse (20 ± 5°C). The inoculation by the pathogen was made by adding 5 ml of 2.10^5 zoospores /ml suspension directly into the pot around the root plants.
Disease severity and root, shoot fresh weight, root and shoot height were determined 15 days after inoculation. Disease severity for seedlings was rated using a 4-class scale: 0, healthy or no apparent discoloration; 1, less than 25% discoloration of the root; 2, 25–50% discoloration of the root; 3, 50–75% discoloration of the root; and 4, more than 75% discoloration of the root or dead plants. Each treatment was run with five replications in a completely randomised design and the experiment was repeated two times

Results
In vitro screening of bacteria isolates

A total of 100 bacteria, were isolated from rhizospheric soil. Most of the bacteria tested in dual culture were able to reduce the growth of *P. sojae*.
Four isolates S1, S9, S10 and S11 inhibited the growth of all isolates of *P. sojae* of by more than 50% (Figure 1).
Effect of bacterial inoculation treatment against *P. sojae* under greenhouse
Damage severity on soybean roots was reduced by the treatment with bacteria isolates. The greatest reduction in disease severity was achieved after S1 and S11 application realizing a disease severity index of 2.5 and 1.5 respectively (Figure 2).
Bacteria treated seeds enhance all the growth parameters (root and shoot fresh weight, root and shoot length) in in comparison to the diseased control (Table1).

Conclusion
The isolated bacteria has proven an acceptable efficiency of control of root rot caused by *P. sojae* in soybeans. However, more research is needed in area of understanding the mechanism of biocontrol

Acknowledgements
The authors greatly acknowledge the financial grant from Manitoba pulse and soybeans growers and Agri-Food Research and Development Initiative (ARDI).

Figure 1: *In vitro* inhibition of mycelia growth of *P. sojae*.
S11: reducing *P. sojae* growth by 76%

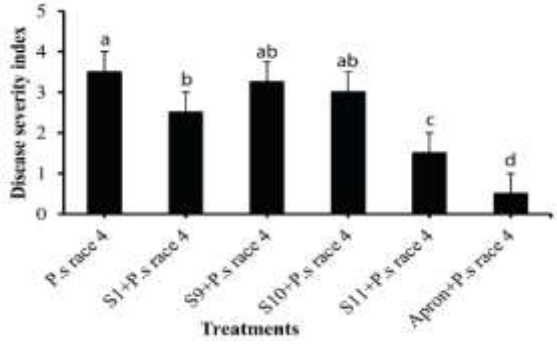

Figure 2. Effect of seed treatment with antagonistic bacteria on severity index of *P. sojae* race 4 on soybean plants (cv. Th32004R), on 15th day after inoculation under greenhouse. Root rot severity of *P. sojae* rated on a1-to- 4 scale. Letters indicate significant differences among treatments according to Duncan test at $P < 0.05$. Each data point represent the average for tree independent replicates with error bars representing the standard errors to the means.

Table 1. Effect of seed treatments with antagonistic bacteria on soybean plant growth parameters, on 15th days after inoculation with *P. sojae* race 4 under greenhouse

Treatments	Shoot height (cm)	Root length (cm)	Fresh shoot weight (g)	Fresh root weight (g)
Control	22.2±2.9^{ab}	20.2±2.9^{ab}	2.1±0.6^{abc}	1.2±0.4^{ab}
P.s race 4	15.5±3.0^{d}	5.3±1.4^{g}	1.2±0.3^{d}	0.5±0.1^{d}
S1	23.7±0.5^{a}	22.7±3.3^{a}	2.2±0.4^{ab}	1.7±0.3^{a}
S1+ *P.s* race 4	20.5±1.2^{bc}	14.0±1.8^{de}	1.7±0.7^{bcd}	0.9±0.3^{c}
S9	22.1±2.5^{abc}	16.5±2.1^{cd}	2.8±0.4^{a}	1.7±0.2^{a}
S9+ *P.s* race 4	21.5±1.0^{abc}	9.8±2.0^{f}	1.6±0.6^{cd}	0.6±0.3^{d}
S10	22.6±1.5^{ab}	19.5±2.1^{bc}	2.3±0.4a	1.6±0.1^{ab}
S10+ *P.s* race 4	20±1.5^{bc}	12.5±2.2^{ef}	1.5±0.1^{bcd}	0.9±0.1^{c}
S11	22.8±2.3^{ab}	15.5±1.3^{de}	2.8±0.3^{a}	1.3±0.1^{b}
S11+ *P.s* race 4	21±0.8^{bc}	14.5±1.3^{de}	2.5±0.4^{abc}	1.2±0.3^{bc}
Apron	20.5±0.5^{bc}	18.5±1.9^{bc}	2.7±0.1^{a}	1.8±0.3^{a}
Apron+ *P.s* race 4	19.0±2.4^{c}	16.2±0.9^{cd}	2.4±0.6^{a}	1.7±0.3^{a}

AP31

INTERACTION OF DRY BEAN MARKET CLASS AND BIOLOGICAL AND CHEMICAL SEED TREATMENTS FOR CONTROL OF SOYBEAN CYST NEMATODE

Zhang, Kaiqi and Chris Gillard

Introduction

Dry bean or common bean (*Phaseolus vulgaris L*.) is an important legume crop for human consumption worldwide (Kutošet al 2003). Soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN) is an important parasitic pest for soybean, and dry bean is an alternate host. Genetic resistance is the primary management option for SCN in soybean, but genetic resistance in dry bean is poorly understood. Mesoamerica market classes (e.g. black bean) have shown higher tolerance to SCN than Andean types (e.g. kidney bean) (Kelly and Cichy 2013; Gepts 1998; Poromarto and Nelson 2009). Potential management of SCN in dry bean with biological controls such as *Pasteuria nishizawae* and *Bacillus firmu*s, and the chemical control fluopyram need to be evaluated.

Objective

The objective of this study are evaluate the tolerance of dry bean cultivar that belong to Mesoamerican and Andean market class to soybean cyst nematode and evaluate the potential of the three seed treatments to manage soybean cyst nematode.

Material and Method

Black bean and kidney bean seed were treated with three seed treatments, bio-controls *Pasturia nishizawae* (Clariva® Syngenta Crop Protection) and *Bacillus firmus* (Votivo® Bayer Crop Science), and a chemical control fluopyram (Ilevo® Bayer Crop Science) at two rates (Table 1). Each experimental unit was inoculated with 4000 eggs of SCN before transplanting the healthy seedlings (>2cm roots). All seedlings were transplanted into cone-tainers (SC10R of Stuewe & Sons Inc) (Figure 1), which was filled with mixed fine sand. Treatments were arranged using a randomized complete block (RCB) design with 6 replications. The experiment was conducted for 30 days in a growth chamber at 27 ^{o}C, with 16/8 hours of light/dark (Figure 2). Plants were watered daily and fertilized at day 14, 17, 21, 24 with 3 ml of 2% solution of 6-11-31.

Result

The dry weight of above ground plant material was measured and the numbers of cysts on plant roots were quantified to calculate female index (FI). FI= (Ni/Ns) ×100, which is Ni=average number of females produced by the SCN on an indicator plant and Ns=average number of females on Lee 74 (Niblack 2005). There is no positive impact of seed treatments or SCN on plants dry weight. There was no difference in FI between the uninoculated and inoculated black bean. FI was similar for the inoculated black bean and the black bean treated with *B. firmus*, fluopyram, and *P. nishizawae.* The inoculated kidney had higher FI than the uninoculated kidney. The inoculated kidney had a higher FI than the uninoculated and inoculated black *B.firmus* or *P. nishizawae* did not reduce FI, compared to the inoculated kidney. Kidney bean treated with *B. firmus* and fluopyram at a high rate have a lower FI than inoculated kidney bean.

Discussion

Currently, with limited data from one experiment, there are not enough accurate data to indicate the impact of seed treatments on FI in black bean. There is also limited data available to detect the impact of SCN inoculation. However, the FI of kidney bean is higher than black bean, which agrees with previous work (Poromarto 2009) that found kidney bean is more susceptible to SCN than black bean. Fluopyram has the potential to reduce early plant vigor (per.comm.L.Bourgois. Bayer CropScience); however, there is no enough evidence of this in our experiment.

AP32

Investigating agronomic practices to remove barriers to Faba bean production in Alberta

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Faba bean is an ancient small-seeded relative of the Chinese broad bean and is an excellent crop for human diets and animal feed around the world. They are high in protein, starch and are a staple in many countries, mostly Ethiopia, where it is eaten as a whole seed but can also be used in the fractioning industry as a food ingredient. Experts are suggesting that using fractionated faba bean ingredients, such as starch, fibre, and protein, in the food market will stimulate a significant expansion of the pulse industry. As animal feed, it can be used as silage or as mature seed ground and included in animal rations. Faba bean is best adapted to wetter soils and does best under cool growing conditions. In the wetter cooler areas of Alberta, where peas have historically been grown, faba bean would represent a great alternative rotational option. As with all pulse crops, faba bean fixes the majority of its nitrogen needs itself, eliminating the addition of nitrogen fertilizer. A major benefit of rotating pulse crops, such as faba bean, with cereal crops is the added benefit to the following crop in soil health, water availability and the nitrogen advantage, as well as the interruption of pest cycles.

Faba bean is a relatively new crop to the majority of Alberta pulse growers and information on the best management practices for this crop is very lacking. Chocolate spot is the main disease affecting faba bean in Alberta, and has the potential to reduce yield by 30-50%. Disease has not reached these levels in Alberta, but as acreage expands and inoculum levels increase, damage due to this disease will also increase. Currently the best management option for Alberta remains unknown. Ascochyta blight is another potential devastating disease threatening faba bean production. This disease was observed in 2014 in Alberta, and may become a major barrier for producers. The most effective tool in managing both of these diseases is foliar fungicide application.Research needs to be performed under Alberta environmental conditions before these diseases inhibit the marketability of this crop. As a pulse crop, faba bean can supply its own nitrogen (N) requirement, making it a valuable crop for rotation in the mostly cereal - canola acreage of the Canadian prairies. However information on other macro- and micro-nutrients is lacking. Anecdotal information from industry agronomists on the use of nutrients such as phosphorus, potassium, sulphur, boron, molybdenum and manganese suggests that addition to a faba bean crop either at seeding or top dressed later results in yield and quality benefits. More comprehensive research across soil zones and agro-climatic areas needs to be performed to support or dis-prove this information.

The objectives of this project included;

Trial 1. To optimize faba bean production and yield through the use of different fungicides to control Chocolate spot (*Botrytis* sp.) and Ascochyta blight in four Alberta climatic zones.

Trial 2. To evaluate macro- and micro-nutrient application to determine advantages for faba bean production in four Alberta soil zones

Field plots were established at four locations across Alberta: Falher, St. Albert, Lacombe and Lethbridge. The trial was set up as a randomized complete block design (RCBD) with four replications. Treatments were applied to two varieties; Snowbird (low or zero tannin type and Malik (tannin type) and seeded at a rate of 45 plants m^{-2}. Data collected included emergence dates and counts, plant height, flowering date, maturity, disease assessment, 1000 TSW, seed yield, and seed quality assessment.

Trial 1. Six fungicide formulations [Lance (boscalid); Acapela (picoxystrobin); Vertisan (penthiopyrad); Priaxor (fluxapyroxad + pyraclostrobin); Headline (pyraclostrobin) and Delaro (prothioconazole + trifloxystrobin)] were applied for management of Chocolate spot (*Botrytis* sp.) and Ascochyta blight. The plots were inoculated to ensure disease presence. There was a total of 16 treatments including inoculated and non-inoculated untreated checks.

Trial 2. Three macronutrients (phosphorus, potassium and sulphur) were applied with the seed and three micronutrients (boron, molybdenum and manganese) were applied at seeding and in crop to compare crop response. One treatment comparing the use of a commercial product Tagteam® was added at seeding as well for comparison. Soil cores were collected before and after the crop and a soil analysis was performed to assess crop usage of each nutrient. There was a total of 24 treatments including 2 untreated control (one for the N-P-K portion of the trial and one for the B-Mo-Mn portion.

The results of these trials showed:

Trial 1.

- Fungicide application resulted in lower disease pressure at all locations when compared to the untreated check
- There were no treatment differences noted for the number of days to maturity within varieties.
- Highest yields were noted in both St. Albert and Lethbridge with the 1.5x rate of Lance (boscalid) fungicide treatment

Trial 2.

- For macronutrients, highest yields were noted when both phosphorus and potassium were added
- Addition of sulphur did not increase yield
- For micronutrients, no differences were noted for days to flower or maturity
- Highest yield was noted in St. Albert and Lacombe with a Precede® treatment formulation from ATP nutrition

The trials resulted in some great information for Alberta pulse producers. The benefits of this project include: (1) increased profitability and diversification of agriculture in Alberta with a renewed high value crop, (2) increased yield and quality of cereal and oilseed crops grown in rotation with faba bean, (3) decreased disease problems in other crops by breaking disease cycles (4) superior food ingredients for food companies and (5) enhanced environmental stewardship and production sustainability with a crop that fixes its own nitrogen supply and reduces producer reliance on fossil fuel-based fertilizers.

AP33

Evaluation of agronomic practices on production of Clearfield red lentil in Alberta

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Lentil is an important global commodity for nutritional and crop rotational purposes. The majority of world consumption, production and trade is in red lentil. It is the second largest pulse crop grown in Canada and this country is one of the major exporters. Growing climatic concerns in other lentil growing areas of the world has created a significant growth opportunity for an increase in Canadian exports. Lentil is an important food staple as they are a vital source of dietary protein and carbohydrates. They are high in fibre, saturated fat free, sodium free, an excellent source of folate, good sources of vitamins, and gluten free. Red lentil, as with any pulse crop, can supply the majority of its nitrogen requirement by fixing nitrogen from the air when inoculated with bacterial inoculants. This makes it a valuable crop for rotation in the mostly cereal - canola agricultural acreage of the Canadian prairies

Red lentil is a relatively new crop to the majority of Alberta pulse growers. Although, there has been extensive research on lentil in Saskatchewan, this research has not taken place in Alberta due low acreage (until recently). High demand and coincidentally high prices have pushed Alberta farmers into taking a serious look at lentil as a viable crop for their rotation. Issues for sustainable lentil production in Alberta are related to grower knowledge and resulting success. Pulses supply their own nitrogen requirements but antidotal information from industry has suggested that the addition of starter nitrogen may be beneficial. Information on the effects of nitrogen, the addition of supplemental rates and the rhizobial interaction to enhance lentil growth, is inadequate at this time. Herbicide use can be of concern as well. There are few broad-leaf herbicides available providing only a narrow window of application. Information on the best weed control products and the effect on the number and efficiency of nodules produced is limited. The optimal seeding rate is another issue. The commonly used seeding rates for lentil were originally recommended for large green types. With seed costs being very high and growers using different rates, success varies.

The earlier maturity advantage of red lentil, coupled with the imidazolinone herbicide tolerance should allow for expansion into other agro-climatic zones within Alberta. Providing research data that addresses key production concerns and extending that information will facilitate growth. This project provided enhanced understanding of key growing aspects of this crop and local information which will lead to grower confidence in growing red lentil in Alberta.

The objectives of this project included;

Trial 1. To determine the most effective nitrogen application rate for Clearfield red lentil to optimize nodulation, yield and overall performance in Alberta soil zones.

Trial 2. To determine the optimal seeding rate of Clearfield red lentil for production in five Alberta soil zones.

Trial 3. To optimize Clearfield red lentil competitiveness and productivity through the use of different “imidazolinones” herbicide formulations and to determine the effect on nodulation and disease severity.

Field plots were established at five locations across Alberta: Falher, St. Albert, Stettler, Brooks and Lethbridge. The trial was set up as a randomized complete block design (RCBD) with four replications. Treatments were applied to two varieties; CDC Maxim CL and CDC Dazil and seeded at a rate of 110 plants m^{-2}. Data collected included emergence dates and counts, plant height, flowering date, maturity, inter-node length, nodulation assessment, lodging, disease assessment, 1000 TSW, protein determination, seed yield and an official seed grade assessment.

Trial 1. Five fertility treatments [0, 15, 30, 45 and 60N kg ha^{-1}] were applied to lentil plots both inoculated with granular rhizobium bacteria and non-inoculated.

Trial 2. The effect of five seeding rates (targeting 40, 80, 120, 160, and 200 plants m^{-2}) were tested. All treatments will receive granular rhizobium inoculants when seeded.

Trial 3. Four commonly used herbicides: Solo (imazamox), Odyssey (imazamox + imazethapyr), Odyssey DLX (imazamox + imazethapyr + tepraloxydim) and Ares (imazamox + imazapyr) were applied and a hand weeded treatment was used for a control check (no herbicide) for comparison. All treatments will receive granular rhizobium inoculants when seeded.

The results of these trials showed that:

Trial 1.

- Inoculation with rhizobia resulted in higher red lentil yield regardless of nitrogen application, although usually not significant.
- Native *rhizobia* is not adequate and high levels of nitrogen does not compensate.
- Higher levels of nitrogen negatively affected nodulation of red lentil.
- Starter nitrogen amounts between 15–30 kg ha^{-1} were beneficial, but above 30 kg ha^{-1} negatively affected lentil growth, nodulation and yield.

Trial 2.

- Plants seeded at 40 plants m^{-2} were short and had thick stems.
- Plants seeded at 200 plants m^{-2} were tall and had thin stems.
- Higher seeding rates resulted in higher yield up to160 plants m^{-2}.
- Above 120 plants m^{-2} yield increase depended on available moisture.

Trial 3.

- All imidazolinone herbicide formulations worked at effectively controlling weed pressure.
- There was no significant damage to nodulation, and yield was not decreased.
- The most effective herbicide formation differed depending on soil type and climatic conditions.

The trials resulted in some great information for Alberta pulse producers. The benefits of this project include: (1) increased profitability and diversification of agriculture in Alberta with a new high value crop, (2) increased yield and quality of cereal and oilseed crops grown in rotation with lentil, (3) decreased disease problems in cereal and oilseed crops by breaking disease cycles (4) superior food ingredients for food companies and (5) enhanced environmental stewardship and production sustainability with a crop that fixes its own nitrogen supply and reduces producer reliance on fossil fuel-based fertilizers.

AP34

DIVERSIFYING CROP ROTATIONS WITH PULSES ENHANCES SYSTEM PRODUCTIVITY

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Agriculture in rainfed dry areas is often challenged by inadequate water and nutrient supplies. Traditionally, summerfallowing is used to conserve rainwater and promote the release of nitrogen via the N mineralization of soil organic matter, but summerfallowing is shown to have environmental consequences. In recent years, pulses have been used to replace summerfallowing, but it is unknown whether the pulse alternative would provide similar benefits in soil water conservation and nutrient supplies as does summerfallowing. Here, we quantified soil water, soil residual N, and the system productivity of pulse-based rotation systems in comparison with wheat or summerfallow-based systems.

Materials and Methods

Field experiments were conducted at the AAFC's Swift Current Research Centre. The 3-yr crop sequences were conducted from 2005 to 2011 for five cycles. Spring wheat in the 1st year and durum wheat in the 3rd year were planted uniformly in the rotation; in Year-2, various pulses and cereals were no-till planted in the Year-1 stubble plots with a summerfallow as the check. Soil water and residual soil N were measured to 1.2 m at five depths both pre-seeding and post-harvest in each year at each plot. The test years were categorized as dry, normal or wet based on the growing-season (May-August) precipitations.

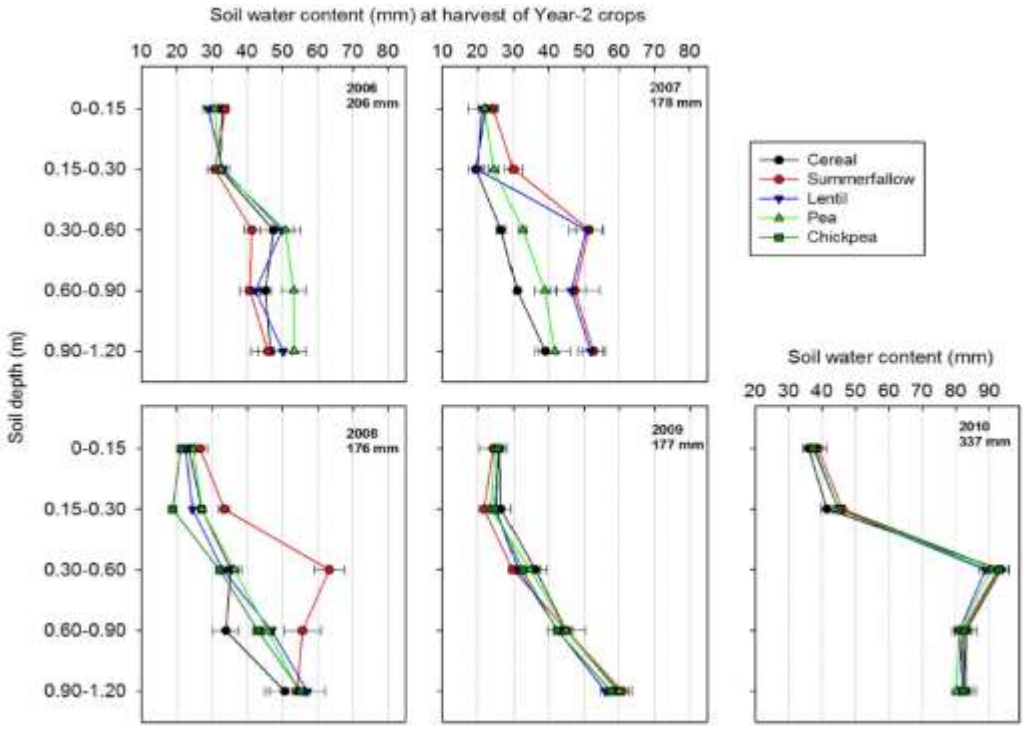

Fig. 1 Soil water remaining at the various depths of the 0–1.2 m soil profile at the harvest of the Year-2 crops.

Results and Discussion

In 2006, 2007, 2008, 2009 and 2010, summerfallowing conserved 48.5, 52.3, 52.1, 29.7 and 23.4 mm of water in the 0-1.2 m soil profile, suggesting that about 79% of the precipitation during the growing season was lost without being conserved. Across the 1.2 m rooting zone, the amount of water remaining in the soil increased with soil depth and the magnitude of the change varied from year to year (Fig. 1). Summerfallow and the cropped treatments had similar soil water distribution patterns in 2006, 2009, and 2010. However, in 2007 and 2008, summerfallow had more water remaining in the top 60 cm depth than the cropped treatments measured at post-harvest. Both pulse- and summerfallow-based systems enhanced soil N availability, but the pulse system did so through the biological N_2-fixation, whereas the summerfallow-system relied on 'mining' soil N at the expense of soil organic carbon (Fig. 2). The largest change in soil N from post-harvest to the following spring planting time occurred in the fall 2007 to spring 2008 period when the summerfallow fields lost 63.9 kg N ha^{-1}, while the fields after pulses increased soil N by 25.3 kg ha^{-1}. In the 3-yr cropping cycle, the pulse system increased total grain production by 35.5%, improved protein yield by 50.9%, and enhanced fertilizer-N use efficiency by 33.0% compared with the wheat-based system with one summerfallow phase in four years.

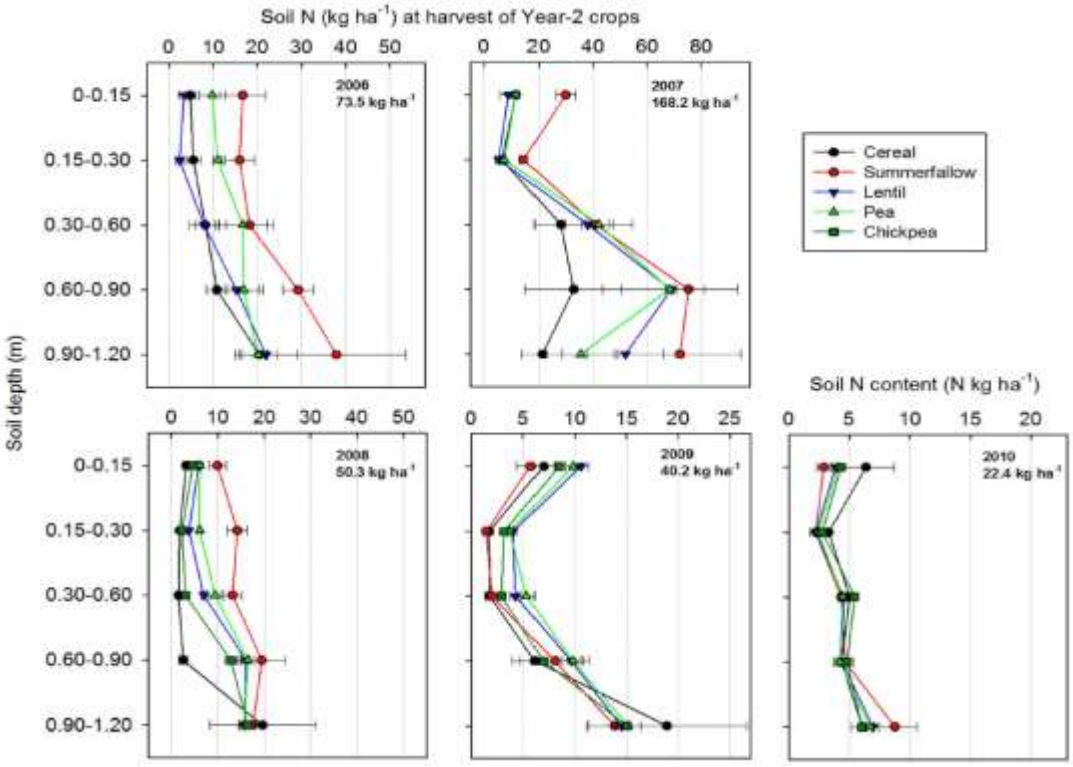

Fig. 2 Residual soil N at the various depths of the 0-1.2 m soil profile measured at the harvest of Year-2 crops.

Conclusion

Diversifying cropping systems with pulses improves soil water conservation, enhances residual soil N availability, and increases system productivity. Pulse-based diverse rotation can serve as an effective approach for increasing precipitation use efficiency and promoting residual soil N in crop production.

Nutrition and Food

Keynote Speaker – Abstract

ACUTE EFFECTS OF EXTRUDED PULSE PRODUCTS ON GLYCEMIC RESPONSE, APPETITE, AND FOOD INTAKE IN ADULTS

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Studies have shown that when whole pulses are consumed alone or within a meal they can improve post-prandial glycemia and satiety control. However, whether pulse ingredients retain the health benefits of whole pulses when consumed in extruded food products is unclear. Two acute trials were recently completed and indicate that post-prandial glycemic effects are dependent upon ingredient type. The objective of these trials was to examine the effects of extruded pulse products on food intake at an *ad libitum* pizza meal at 120 min, as well as appetite and blood glucose responses pre-pizza (0-120 min) and post-pizza meal (140-200 min). The first trial investigated the effects of extruded pulse snacks containing 40% pulse flour and 60% corn flour, compared to a 100% corn control. Pinto bean and chickpea snacks led to lower pre-pizza blood glucose area under the curve (AUC) compared with control, whole yellow pea and green lentil snacks. There were no effects on glycemic control following the pizza meal. The second trial investigated the effects of replacing oats with yellow pea fractions in extruded cereals, compared to a 100% oat control. Pre-meal blood glucose AUC was lower following the pea protein, and protein combined with fibre and/or starch compared to pea starch alone and lower after the pea protein, starch and fibre cereal compared to control. In contrast, post-meal blood glucose AUC was higher following the pea protein, starch and fibre cereal compared to control. Neither study identified effects on appetite or food intake. Data from these trials indicate that effects on post-prandial blood glucose may be dependent upon pulse ingredient type. It also supports the use of specific pulse ingredients in foods designed to improve glycemic control.

NO1

BULK PHYSICAL PROPERTIES OF STORED BLACK AND WHITE BEANS

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Physical properties of stored pulse seeds are important parameters in design of storage and handling facilities and equipment. The following physical properties of black (turtle) and white (navy) beans (*Phaseolus vulgaris* L.) with 12%, 14%, 16%, and 18% moisture contents (wet basis) were measured under room conditions: germination, size (dimension), thousand kernel mass, bulk density, kernel (true) density, filling and empty repose angle, and coefficient of friction against four structural surfaces (galvanized steel, plywood, steel-trowelled concrete, and wood-floated concrete). Germination of the newly harvested beans was higher than 97%. Moisture content significantly influenced their kernel and bulk densities, while it did not affect the repose angle. The emptying angle was significantly larger than the filling angle of repose. Coefficient of friction against structural surfaces significantly increased with increase of moisture content and significantly affected by the surface materials. The largest coefficient of friction was against the wood-floated concrete followed by the steel-trowelled concrete, galvanized steel, and plywood surface for both bean types.

NO2

ACUTE EFFECTS OF LENTIL FRACTIONS ON SATIETY AND GLYCEMIC RESPONSES BEFORE AND AFTER A MEAL IN HEALTHY YOUNG MEN

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Background: Pulses are low glycemic foods high in protein, dietary fibre, and resistant starch and that suppress appetite and postprandial glycemia (PPG). Regardless, a majority of Canadian consumers do not include pulses as part of a regular diet despite their demand for healthier food options. One potential approach by the food sector for meeting this demand is to utilize pulses as value-added ingredients in commonly consumed foods. However, there is a need for research studies to examine which component of pulses (protein, starch, dietary fibre) is responsible for delivering health benefits such as appetite suppression and reduction in PPG.

Methods: Lentils are low glycemic foods, however, it is not clear which components are responsible for this effect. The present study examined the effects of commercially prepared lentil macronutrient concentrates (i.e. fiber, starch and protein) used as value-added food ingredients on subjective appetite, blood glucose (BG) and insulin before and after a pizza meal consumed by healthy young men. Two randomized, cross over, repeated measures experiments were conducted. Forty-eight healthy body-weight young males consumed iso-volumetric (300 ml) servings of tomato soup alone (control) or with added lentil concentrates of protein (75% or 55%), starch (60% starch) or fiber (55% fiber). Additions provided 20 g of protein, starch or fiber to the soup. A pizza meal of fixed amount (12 kcal/kg body weight) was served either 30 (exp-1) or 120 minutes (exp-2) later. Subjective appetite, BG and insulin were measured at intervals from baseline to 170 min (exp-1) and 200 min (exp-2). The lentil flours were analyzed to ensure consistency for size using light scattering. Additionally, starch in lentil starch flour was analysed by differential scanning calorimetry and scanning electron microscopy to relate starch microstructure to PPG. It has been shown that BG levels are influenced by the properties of food ingredients and in particular starch microstructure, which remains to be studied in a variety of pulse types including lentils.

Results and Discussion: All lentil fractions used in this study were finely ground (particle size < 200 μm). In both experiments post-treatment BG and insulin increased after lentil starch compared to the control and other lentil fractions ($P<0.0001$). In exp-1, within meal increase in glucose was lowest after the 55% protein and starch concentrates. Post-meal (50-170 min) BG was lower following both protein soups compared to control ($p<0.05$, Fig.1A). Although there were no significant differences among the treatments in cumulative (0-170 min) incremental area under the curve (iAUC) for BG, lentil protein at both concentrations lowered iAUC by 17% (at 75% protein) and 19% (at 55% protein) in comparison to control with no disproportionate increases in insulin. In exp 1, post-treatment (0-30 min) serum insulin levels were higher at 30 min after lentil starch. The post-meal (50-170 min) insulin response peaked at 80 min with no differences among the treatments. In addition, post meal appetite scores were lower after both lentil protein soups (Fig. 1B). The ability of lentil proteins to suppress BG and appetite was not observed when meal time was extended from 30 min to 120 min (exp 2). With the exception of higher post-treatment BG and insulin values following lentil starch, there were no post-treatment (0-120 min) or post-meal (140-200 min) effects on BG, appetite or insulin in exp-2.

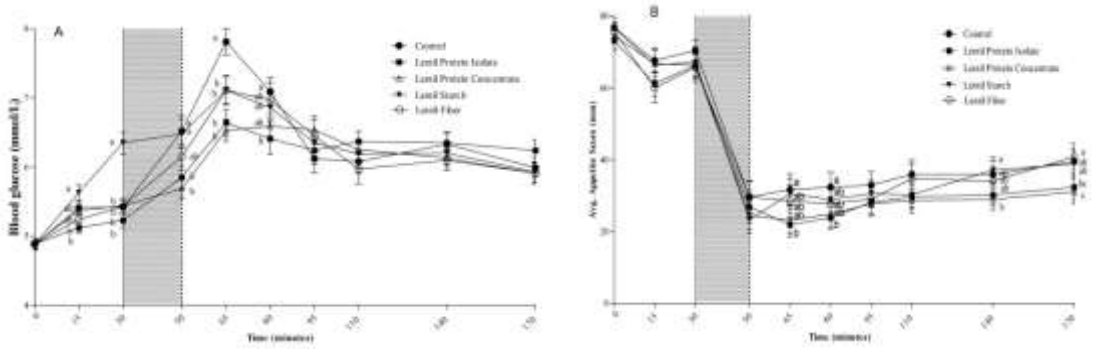

Figure 2. Effect of treatments on (A) blood glucose concentrations and (B) subjective appetite over time. Values are means, with their standard errors represented by vertical bars (n=24)
[a,b] Mean values with unlike letters were significantly different at each measured time (P<0·05, one-way ANOVA, Tukey–Kramer post hoc test).

The effect of proteins on glucose metabolism depends on a variety of factors, including the amino acid composition, digestion kinetics of proteins and utilization of amino acids released in the gastrointestinal tract. Additionally, the rate of protein digestion relates to the ability of proteins to suppress PPG. Therefore, rapidly digested proteins, such as whey, would exert a more acute effect. It is plausible that the time-course of lentil protein hydrolysis follows a similar pattern to whey as both are comprised of globular proteins. This may explain why the effects of lentil protein on PPG in this study were not observed when meal time was extended from 30 min (exp 1) to 120 min (exp 2). In the present study, lentil starch concentrate led to higher post-treatment BG levels. Although the carbohydrate content was naturally higher this may also be due to the form of starch present. It has been shown that intact (native) starch granules are resistant to digestion and therefore elicit lower glycemic responses in comparison to gelatinized or partially hydrolysed starch where the native structure is lost during cooking or processing. The peak gelatinization temperature of starch from lentil starch concentrate was measured at 67 ^{o}C, which means that exposing the granules to a higher temperature would result in a loss in crystalline structure and increased digestibility. Moreover, there was heterogeneity in starch morphology of lentil starch granules where some were intact (Fig. 2A) while others showed evidence of structural degradation/surface erosion (Fig.2 B, C). This would lead to more efficient hydrolysis of starch and higher PPG in line with the *in vivo* changes we observed in BG.

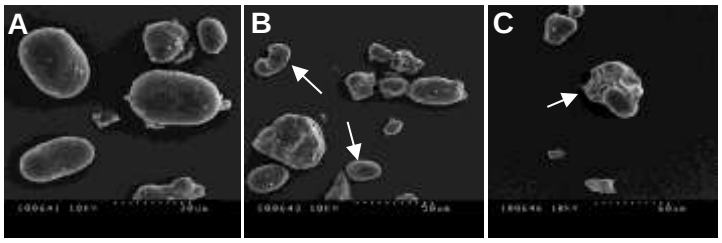

Figure 3. Morphology of lentil starch granules showing their native, ellipsoid (A) and partially degraded structure indicated by white arrows (B, C)

Conclusion: Lentil flours used as value-added ingredients in foods may provide benefits by modulating postprandial glycemia and appetite.

NO3

THE ROLE OF A PULSE-BASED DIET AND AEROBIC EXERCISE ON REPRODUCTIVE AND METABOLIC MEASURES IN WOMEN WITH POLYCYSTIC OVARY SYNDROME: A RANDOMIZED CLINICAL TRIAL

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Background: Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder associated with metabolic syndrome and anovulatory infertility in women of reproductive age. While lifestyle intervention is the first-line treatment of PCOS, optimal dietary composition is unclear.

Aim: To compare the influence of a pulse-based diet with the standard therapeutic lifestyle change (TLC) diet on metabolic and reproductive features of PCOS.

Methods: 95 women with PCOS (18-35y) were enrolled in a 16-wk intervention with 30 completing the pulse-based diet and 31 the TLC diet while participating in a supervised exercise program.

Results: Both groups improved fasting insulin and D-lactate levels, body mass index, blood pressure, trunk fat mass, %fat, %appendicular skeletal muscle mass, total cholesterol, and bilateral antral follicle counts, along with a trend towards reduced androgen levels, without differences between groups. However, the pulse-diet significantly reduced cholesterol to HDL ratio, LDL, TG, and increased HDL concentrations compared to the TLC diet ($P<0.05$).

Conclusion: Both diets yielded clinically important improvements in metabolic and reproductive features of PCOS, while the pulse-diet was superior at improving lipid profile.

NO4

RED KIDNEY BEANS AND LENTILS INDUCE ACUTE VASORELAXATION IN HEALTHY ADULTS

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Consumption of pulses (dried beans, peas, chickpeas, and lentils) over many weeks can decrease cardiovascular disease risk. However, it is unknown whether pulses can affect blood vessels within a few hours of consumption. Since dried beans and lentils can improve arterial stiffness, we hypothesized that they would yield greater improvements in vascular tone (an acute indication of endothelial function) compared to a rice control. We therefore compared different bean varieties (black, navy, pinto, and red kidney beans), lentils and white rice for their acute effects on blood pressure and vascular tone in healthy individuals. Vascular measurements were obtained in 8 healthy adults (4 men/4 women; 37±4 years; body mass index of 24.3±0.9 kg/m^2) before and at 2 and 6 hours after the pulses were eaten. Vasorelaxation as shown by reduced pulse wave reflection magnitude was observed 6 hours following consumption of red kidney beans and lentils compared to 2 hours post consumption (53.3±3.3% vs 61.1±3.7% and 55.5±4.4% vs 62.1±2.1%, respectively; $p<0.05$). Reflection magnitude did not change between baseline and 2 hours. Additionally, neither blood pressure nor arterial stiffness (determined by pulse wave velocity, augmentation index and augmentation pressure) changed within or between the groups over 6 hours. These latter results were not surprising since hypertension and arterial stiffness are conditions that develop over time (weeks to months). Overall, red kidney beans and lentils elicited a positive effect on the tensile properties of blood vessels, and this acute response may provide insight for how pulses modify vascular function.

NO5

COMPARISON OF BEANS AND PEAS FOR CHOLESTEROL-LOWERING: A RANDOMIZED CLINICAL TRIAL IN ADULTS WITH MILD HYPERCHOLESTEROLEMIA

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Although beans have been investigated for their cholesterol-lowering properties there is a relative absence of studies on dried peas. The objective of this multi-site randomized clinical trial was to compare the effects of consuming foods containing Beans or Peas to Rice (control) on blood cholesterol in men and women with mild hypercholesterolemia but not taking any cholesterol-lowering medications. Participants (n=180) consumed the study foods (a selection of 5 items containing 120 g beans or peas or rice per serving) five times per week for 6 weeks as part of their usual diet. The study foods were provided frozen and contained either Beans (black, great northern, pinto, navy, mixture of all 4), Peas (yellow, green, mixture of both) or Rice. After 6 weeks, the Beans group, but not the Peas group, had significantly lower LDL-cholesterol and total cholesterol compared to Rice. These results indicate that pulse type will have to be specified in a cholesterol-lowering health claim submission for pulses.

NO6

NUTRIENT INTAKES OF MANITOBA CHILDREN AND YOUTH: A POPULATION BASED ANALYSIS BY PULSE AND SOY CONSUMING STATUS

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Poor eating habits among children are associated with negative health outcomes such as increased risk of childhood obesity, type 2 diabetes and increased risk of developing adult obesity. The objective of this study was to use pulse/soy consumption as an indicator to evaluate the eating profile of young Manitobans. This analysis used data from the Canadian Community Health Survey Cycle 2.2 (CCHS 2.2) conducted by Statistics Canada and methods similar to those used in previous analysis of bean, pea and lentil consumption patterns. The CCHS 2.2 was completed in 2004 and targeted respondents from all age groups living in the ten provinces. The main objectives were to gather information on the nutritional status of Canadians and to estimate the distribution of dietary intake in terms of foods, food groups, dietary supplements, nutrients and eating patterns among a representative sample of Canadians at national and provincial levels using a 24-hour dietary recall. Respondents were restricted to Manitoba residents aged 2 to 18 years (n = 1840). Consumers were identified as individuals who reported eating at least 1 pulse/soy product during their recall. On any given day, 8.2% of Manitobans reported consumption of pulses/soy. Intakes of fibre, protein, magnesium, and zinc were higher in consumers only when expressed relative to total caloric intake. Consumers also reported increased intakes of meat and alternatives. Total intakes of vitamin D, fibre, and fruit and vegetable consumption were low among all groups. Sodium intakes in both groups were high when compared with levels recommended by health professionals. These results indicate that there are many dietary issues affecting Manitoba children, suggesting the need for more research targeting dietary habits of children and youth, the quality of the food supply, and effective strategies in nutrition education.

Macronutrient, Micronutrient and energy intakes[a] per day of Manitoban youth 2-18 years based on 1 day dietary recalls from the Canadian Community Health Survey, Cycle 2.2

	Overall [n=1840]	Non-Consumers [n=1690]	Pulse/Soy Consumers [n =150]
Food amount [g]	2332 ± 59	2335 ± 49	2294 ± 341
Energy [kcal]	2123 ± 71	2136 ± 93	1964 ± 246
Carbohydrate [g]	294 ± 12	296 ± 16	262 ± 42
Carbohydrate per 1000 kcal [g]	140 ± 2	140 ± 2	133 ± 6*
Fibre [g]	13.7 ± 0.3	14.1 ± 0.3	14.2 ± 2.0
Fibre per 1000 kcal [g]	6.6 ± 0.3	6.5 ± 0.2	7.6 ± 0.6*
Sugar [g]	140.0 ± 9.1	142.1 ± 12.2	117.3 ± 35.1
Total Fat [g]	74.3 ± 2.0	75.2 ± 3.0	69.1 ± 7.2
Total Fat per 1000 kcal [g]	34.2 ± 0.5	34.1 ± 0.7	35.0 ± 1.5
Saturated Fatty Acid [g]	29.1 ± 0.7	26.4 ± 1.4	24.3 ± 2.7
Saturated Fat per 1000 kcal [g]	10.8 ± 0.1	12.1 ± 0.4	12.0 ± 0.6
Monounsaturated Fatty Acid [g]	28.1 ± 2.6	29.1 ± 0.9	28.4 ± 2.6
MUFA per 1000 kcal [g]	13.3 ± 0.3	13.3 ± 0.4	14.1 ± 0.7
Polyunsaturated Fatty Acid [g]	11.9 ± 0.3	12.2 ± 0.3	11.3 ± 1.3

PUFA per 1000 kcal [g]	5.5 ± 0.2	5.5 ± 0.2	5.7 ± 0.3
Cholesterol [mg]	239 ± 19.3	225 ± 10	240 ± 19
Cholesterol per 1000 kcal [mg]	108 ± 4	107 ± 6	131 ± 32
Protein [g]	75.6 ± 2.0	75.1 ± 2.5	79.2 ± 8.2
Protein per 1000 kcal [g]	36.7 ± 0.7	36.2 ± 0.6	41.3 ± 2.0*
Vitamin D[mg]	6.5 ± 0.2	6.5 ± 0.2	6.4 ± 1.7
Vitamin D per 1000 kcal [mg]	3.2 ± 0.1	3.2 ± 0.1	3.5 ± 0.4
Vitamin C [mg]	131 ± 7	132 ± 7	110 ± 30†
Vitamin C per 1000 kcal [mg]	67 ± 5	67 ± 5	55 ± 11**
Thiamin [mg]	1.7 ± 0.0	1.7 ± 0.1	1.7 ± 0.1
Thiamin per 1000 kcal [mg]	0.8 ± 0.0	0.8 ± 0.0	0.9 ± 0.1
Riboflavin [mg]	2.2 ± 0.1	2.2 ± 0.1	2.0 ± 0.2
Riboflavin per 1000 kcal[mg]	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
Niacin [mg]	33.0 ± 0.7	33.2 ± 0.8	34.1 ± 3.7
Niacin per 1000 kcal [mg]	15.9 ± 0.4	15.8 ± 0.4	17.5 ± 1.2
Vitamin B_6 [mg]	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.2
Vitamin B_6 per 1000 kcal [mg]	0.7 ± 0	0.7 ± 0.0	0.8 ± 0.1
Vitamin B_{12} [mg]	3.8 ± 0.2	3.8 ± 0.2	3.9 ± 2.9
Vitamin B_{12} per 1000 kcal [mg]	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
Folic Acid [µg]	134 ± 15	136 ± 16	127 ± 11
Folic Acid per 1000 kcal [µg]	66 ± 6	66 ± 6	67 ± 9
Folate [from food in dietary folate equiv.] [µg]	426 ± 21	428 ± 26	400 ± 47
Folate per 1000 kcal [µg]	206 ± 4	205 ± 5	207 ± 12
Calcium [mg]	1078 ± 45	1080 ± 58	1062 ± 155
Calcium per 1000 kcal [mg]	518 ± 7	517 ± 8	519 ± 7
Phosphorus [mg]	1358 ± 41	1357 ± 54	1365 ± 164
Phosphorus per 1000 kcal [mg]	652 ± 7	648 ± 8	708 ± 29†
Magnesium [mg]	268 ± 5	267 ± 6	273 ± 38
Magnesium per 1000 kcal [mg]	130 ± 3	129 ± 3	142 ± 7*
Iron [mg]	13.9 ± 0.6	14.1 ± 0.8	14.2 ± 2.4
Iron per 1000 kcal [mg]	6.7 ± 0.1	6.7 ± 0.1	6.7 ± 0.5
Zinc [mg]	10.1 ± 0.3	10.3 ± 0.4	11.2 ± 0.9
Zinc per 1000 kcal [mg]	4.9 ± 0.1	4.8 ± 0.1	5.5 ± 0.3*
Sodium [mg]	3028 ± 62	3027 ± 86	3031 ± 588
Sodium per 1000 kcal [mg]	1445 ± 48	1436 ± 48	1568 ± 80
Potassium [mg]	2669 ± 56	2669 ± 54	2762 ± 387
Potassium per 1000 kcal [mg]	1303 ± 44	1297 ± 45	1397 ± 68
Grain Products [servings]	6.1 ± 0.4	6.1 ± 0.5	5.7 ± 0.6
Vegetable and Fruit Products [servings]	3.8 ± 0.1	3.8 ± 0.1	3.3 ± 0.5
Milk and Alternatives [servings]	2.5 ± 0.1	2.5 ± 0.2	2.4 ± 0.4
Meat and Alternatives [servings]	2.9 ± 0.1	2.9 ± 0.1	4 ± 0.4***

[a] Intakes ± SD, Note: Comparing to non-consumers * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, †$0.1 < p < 0.05$

NO7

TEXTURAL PROPERTIES AND OIL UPTAKE OF FALAFEL PREPARED FROM CHICKPEA AND FABA BEAN: A HEALTHY, PULSE-BASED MEDITERRANEAN FOOD

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Introduction

Falafel is one of the most popular street foods in Mediterranean countries. It originated in Pharaonic Egypt, and are eaten by *Copts* (the christians of Egypt) as a meat substitute during *Lent* (religious observance of 6 weeks). These deep-fried balls are traditionally made from soaked but uncooked chickpeas and/ or dehulled faba beans and usually served with pita bread, and seasoned with spices, vegetables and tahini sauce(1). This dish provides high level of protein, complex carbohydrates, phenolic compounds, vitamins, minerals, fat and fiber. The present study investigated the chemical composition, oil uptake and sensorial properties of falafel with different formulations, and using innovative pulse ingredients such as precooked flours that can improve the convenience of preparation. Our study also highlights the potential use of falafel as a healthy and balanced fast food that can be a meat alternative.

Keywords*:* Chickpea, faba bean, falafel, frying, meat alternative.

Objective

The objectives of this study were to examine the oil absorption, textural properties and contents of vicine and convicine of falafel made from raw or treated (tempered and infrared heated chickpea or dehulled faba bean) grit and compare with falafel made by the traditional method from soaked but uncooked ground seeds.

Materials and methods

Three falafel mixtures were prepared as following: 1- The control was prepared using a traditional method from soaked chickpea and/ or dehulled faba bean seeds in water (16h), followed by chopping in a food processor with spices (10.5%). 2- Raw seeds grit, or precooked seeds grit (tempered for 16 h to 16 % moisture, and infrared heated to 120-125 ºC surface temperature). The coarse grits so prepared were mixed with spices and water (1:1.1; w:w) and kept at room temperature for 30 min. The paste was then deep fried in canola oil at 180 ºC. Physical and chemical analyses were conducted according to AOAC (2000) methods, vicine and convicine levels were measured after extraction with NaOH and by UPLC, and the textural properties were evaluated by TMS-Pro Texture Press.

Results

The results of moisture (before and after frying) and oil uptake of fried product show that the oil retention increased with water loss upon frying of all samples (Table 1). The oil holding capacity of the paste (before frying) prepared from chickpea seeds in the traditional method was higher compared to the paste prepared with raw or precooked chickpea grit. The fried product of raw, unheated chickpea grit retained much less fat (16 %) than falafel made from heat treated chickpea grit or soaked ground seeds (~22 %). The falafel paste made from raw or precooked faba bean grit retained less fat (~12 %) compared to the paste prepared by the traditional method (~ 21 %). In addition, faba bean falafel had lesser amount of retained fat after frying than those from chickpea. This observation may be related to lesser amount of starch and higher content of protein and fiber in faba bean that may not hold hydrophobic oil(2). Protein content in falafel balls prepared from

chickpea seeds and grit was 10.62-14.05 %, while it was around 22.00 % for falafel prepared from faba beans. Therefore, an enrichment of protein can be achieved when faba bean is used for falafel. The antinutritional components of most concern related to faba bean are vicine and convicine which can cause an acute hemolytic anemia (favism) in humans with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Our results indicated that the vicine and convicine content was reduced by 12-24 % after frying.

Conclusion

Falafel made from raw or precooked grits of dehulled faba bean contained less retained fat compared to traditional falafel made from faba bean or chickpea seeds. Also, frying reduced the level of vicine (24%) and convicine (12%) slightly. Faba bean grit produced falafel with improved textural properties (less hardness and more crispiness) and higher protein content than the ones with chickpea. Detailed investigations on the nutritional and anti-nutritional components and sensory properties will be necessary to fully describe the end-product quality.

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NO8

PROCESSING AND MODIFICATION OF COMMON BEAN POWDERS AS VALUE-ADDED FOOD INGREDIENTS

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Effects of different processing and modification methods on the functional properties and starch digestibility of common bean powders were presented and discussed at the 10th Canadian Pulse Research Workshop 2016. In the first part of our presentation, 25 common bean varieties grown in Michigan were ground into fine (particle size ≤ 0.5 mm) or coarse (≤ 1.0 mm) powders. Both the variety and particle size exhibited an impact on the pasting properties and starch digestibility of the bean powders. For the same variety, fine bean powder showed a higher viscosity but lower resistant starch (RS) content than the coarse counterpart (**Figure 1** and **3A**). Cookies baked from the fine bean powders displayed a smaller diameter, greater height, and larger hardness value than those from the corresponding coarse bean powders (**Figure 2**). Fine bean powders of certain varieties could be used to bake cookies with sizes and hardness values similar to those baked from soft wheat flour. The bean-based cookies displayed greater RS contents (4.9-11.3%) than the soft-wheat-based cookies (0.2-0.8%) (**Figure 3B**). In the second part of the presentation, 2 fine bean powders (Merlot Small Red and Fuji Otebo) were further modified by extrusion with and without the addition of 1%, 3%, and 5% (db) gum arabic or xanthan gum. The presence of xanthan gum increased the viscosity of bean powders, whereas gum arabic decreased the viscosity (**Figure 4**). The addition of the food gums enhanced the water-holding capacity of the bean powders. The food gums did not considerably influence the starch digestibility of the extruded bean powders. Our research has demonstrated how milling and extrusion modification can be effectively utilized to prepare bean powders with versatile functionality and high RS contents.

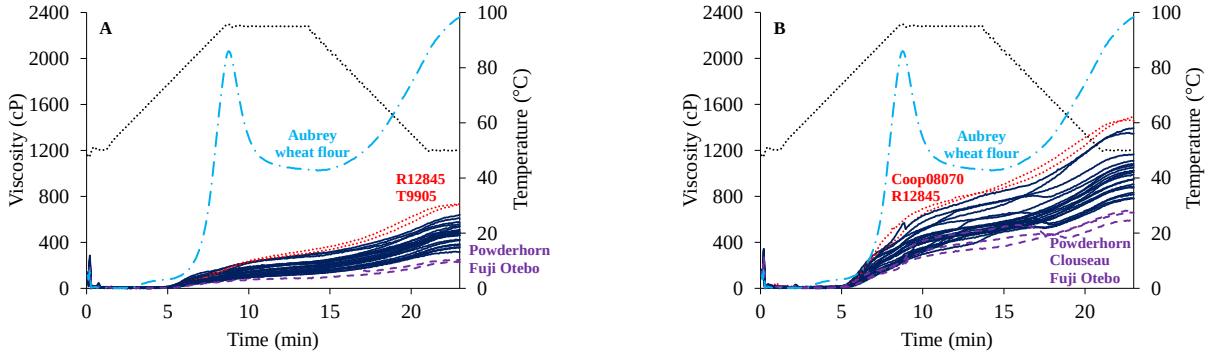

Figure 1. Pasting properties of bean powders of 25 varieties with particle size ≤ 1.0 mm (A) and ≤ 0.5 mm (B) with indications of the highest (red, round dot) and lowest (purple, dash) final viscosities, and pasting properties of Aubrey wheat flour. The analysis was performed using a Rapid Visco Analyzer at 10.6% dry solids content.

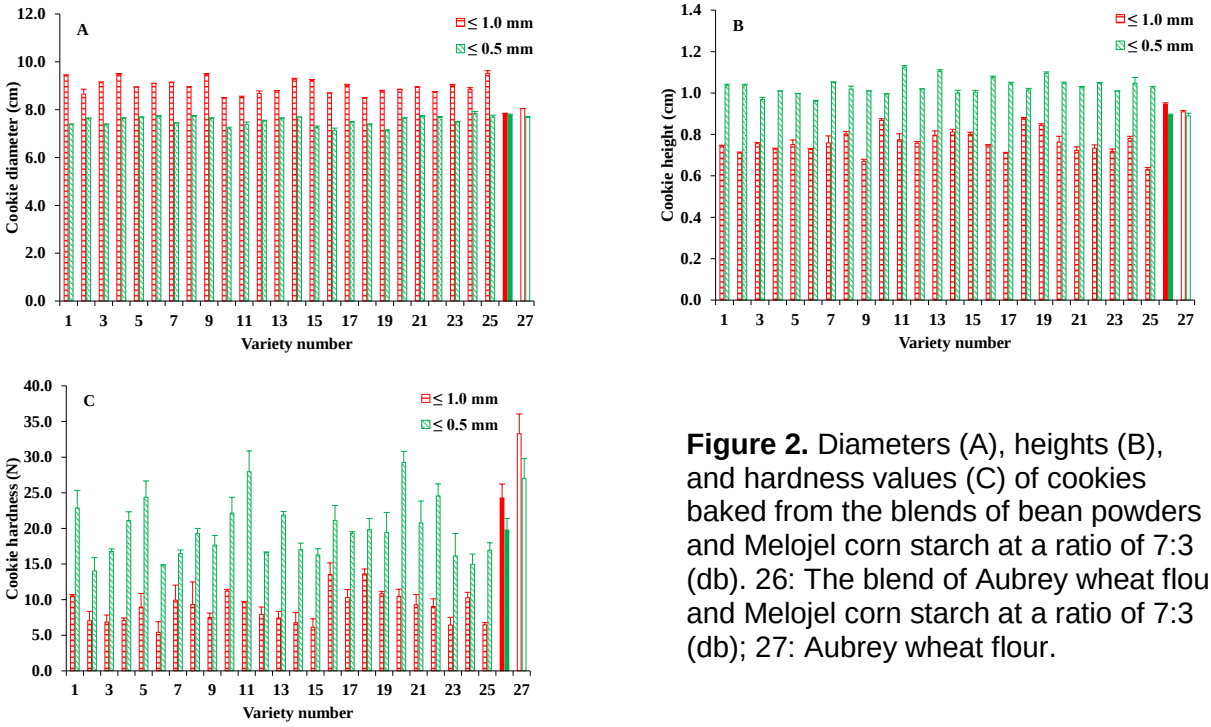

Figure 2. Diameters (A), heights (B), and hardness values (C) of cookies baked from the blends of bean powders and Melojel corn starch at a ratio of 7:3 (db). 26: The blend of Aubrey wheat flour and Melojel corn starch at a ratio of 7:3 (db); 27: Aubrey wheat flour.

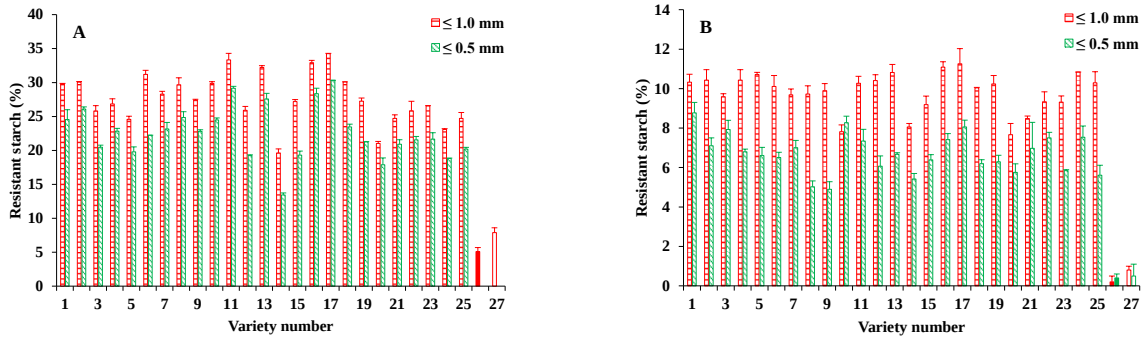

Figure 3. Resistant starch contents of raw bean powders (A) and cookies (B) from 25 bean varieties with particle size ≤ 1.0 mm and ≤ 0.5 mm. 26: The blend of Aubrey wheat flour and Melojel corn starch at a ratio of 7:3 (db); 27: Aubrey wheat flour.

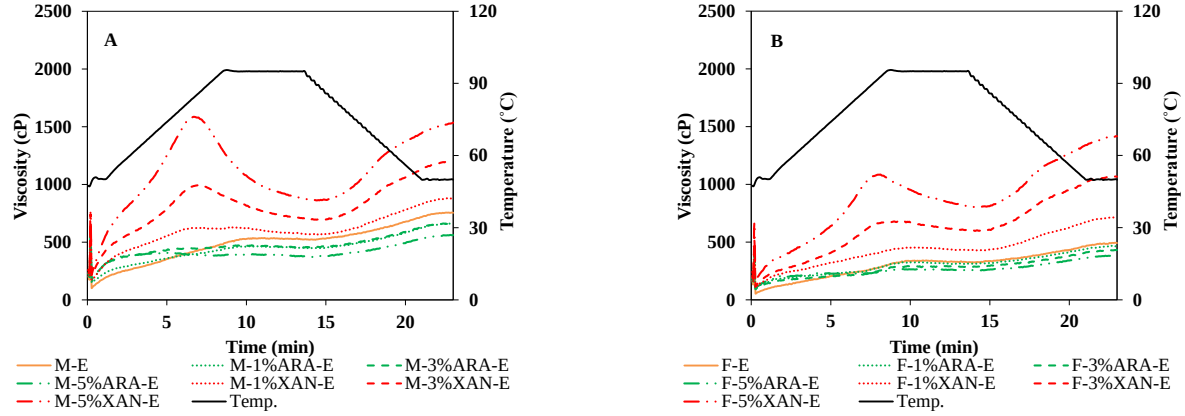

Figure 4. Pasting properties of fine bean powders extruded (E) with 1%, 3%, and 5% (db) gum

NO9

INVESTIGATING THE *IN VIVO* AND *IN VITRO* PROTEIN DIGESTIBILITY RELATIONSHIP IN PULSES AND OTHER PROTEIN SOURCES

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Background

Pulses are very a nutritious legume, with exceptionally high protein content (21-26% by dry weight) relative to other plant-based protein sources. Despite the high protein content, pulses are limiting in the essential amino acids tryptophan, methionine and/or cysteine. When considering protein quality in pulses, it is important to determine whether the amino acids and nitrogen are sufficient and biologically available for normal human metabolism. Several methods exist for the evaluation of protein quality, including the protein efficiency ratio (PER), the protein digestibility corrected amino acid score (PDCAAS), and the digestible indispensable amino acid score (DIAAS). PDCAAS is the most widely adopted method of assessment worldwide, whereas Canada currently uses the PER. Current recommendations from the Food and Agriculture Organization (FAO) of the World Health Organization (WHO) are signifying that DIAAS should replace PDCAAS. All three methods require animals for assessment of protein quality, which makes protein unique in this respect to other nutrients. Additionally, these animal bioassays are lengthy, expensive and may prove challenging to implement for organizations whose policies preclude the use of animal testing.

Alternative *in vitro* protein digestibility (IVPD) methods, such as the static enzymatic pH drop, are a means to bypass *in vivo* challenges. The method, originally developed by Hsu et al. (1977), measures the pH change of a test protein after the addition of an enzyme cocktail containing chymotrypsin, trypsin and protease. Test proteins measured using this technique is calculated using a regression equation originally designed to estimate fecal digestibility. This study investigated the possibility of using *in vitro* derived digestibility values of thirty different proteins, including fifteen pulses, to calculate the *in vitro* protein digestibility corrected amino acid score (IVPDCAAS), and the *in vitro* protein digestibility digestible indispensable amino acid score (IVPDDIAAS). Furthermore, comparisons between all three *in vivo* methods and the *in vitro* method were explored.

Methods

Thirty protein samples, including fifteen pulses, across four studies, were subject to Pearson product-moment correlation coefficient analysis for IVPDCAAS, IVPDDIAAS, PER, PDCAAS and DIAAS. All test subjects were weanling Sprague Dawley rats, randomized and housed in hanging wire bottom cages. Each was fed an isocaloric diet containing 10% protein from the test protein and 10% fat from the test protein and/or vegetable oil. All studies began with habituation to the test protein for 3 days, followed by PDCAAS and PER. Initially 15 g/day of feed for 5 days was offered, after which *ad libitum* consumption remained for the remainder of PER. At the end of 28 days for PER, rats were transitioned to a meal-fed paradigm for five days prior to euthanasia. Feces and ileal contents were collected during PDCAAS and at the end of DIAAS, and measured for nitrogen using LECO combustion. Amino acid composition was determined by acid hydrolysis, performic acid oxidized hydrolysis for sulfur amino acids and alkaline hydrolysis for tryptophan. The static enzymatic pH drop followed procedures previously described (Hsu et al. 1977) with alternations from Tinus et al. (2012). PDCAAS and DIAAS were calculated as previously described (FAO/WHO 1991, 2013), whereas IVPDCAAS and IVPDDIAAS were calculated similarly, with the exception that the digestibility measure was replaced with the *in vitro* measure.

Results and Discussion

Overall, digestibility values measured *in vitro*, may replace digestibility values measured *in vivo* for the calculation of PDCAAS and DIAAS. Strong correlations between PDCAAS and IVPDCAAS (figure 2) as well as DIAAS and IVPDDIAAS (figure 3) were determined for all protein samples (R2 = 0.92, 0.88). A slightly stronger correlation was indicated by pulses alone (R2 = 0.91, 0.92). No significant correlation was observed between PER and values determined via the *in vitro* assay across all the samples or in the pulse subset (figure 1). This suggests that *in vitro* assays, such as the pH drop method, may be a sensitive, inexpensive and ethical replacement method for determining protein digestibility and quality typically assessed via animal bioassays.

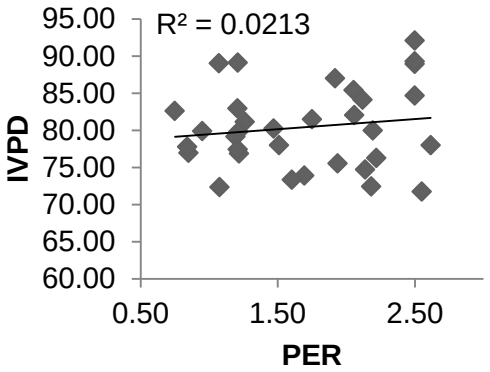

Figure 1. Pearson product-moment correlation coefficient analysis of PER and IVPD

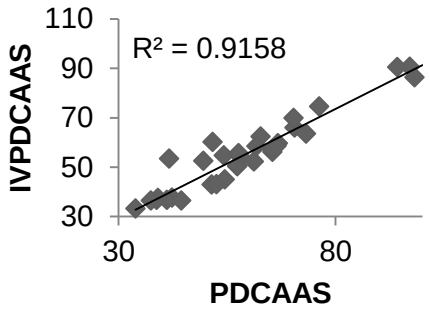

Figure 2. Pearson product-moment correlation coefficient analysis of PDCAAS and IVPDCAAS

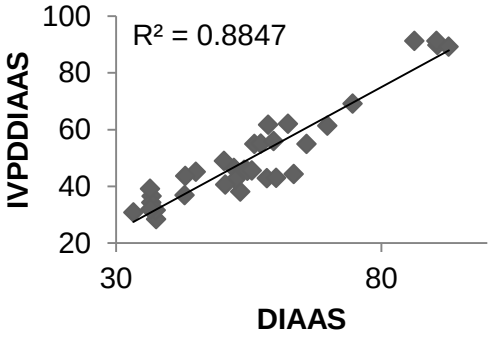

Figure 3. Pearson product-moment correlation coefficient analysis of DIAAS and IVPDDIAAS

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NO10

EFFECT OF BIOPOLYMER MIXING RATIO AND PH ON THE FORMATION OF ELECTROSTATIC COMPLEXES WITHIN MIXTURES OF PEA PROTEIN ISOLATE AND COMMERCIAL PECTIN OF DIFFERENT DEGREES OF ESTERIFICATION

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Protein-polysaccharide interactions play an important role in many food systems in controlling their macroscopic properties and for applications in controlled delivery systems. Polysaccharides can be used in combination with proteins to achieve improved functionality through alterations of the surface chemistry and aggregation behaviour of proteins. As consumer trends change industry is searching for alternatives to protein ingredients from animal-derived sources and soy. Pea protein is emerging as an acceptable alternative based on its nutritional and functional properties, low cost and abundant supply. Gaining knowledge of how it interacts with other food macromolecules (e.g., polysaccharides) and how these interactions could be used to tailor the functionality of pea protein is important in order to broaden its market diversification.

Objective:

The overall objective of this research was to investigate the effect of pH and the degree of methyl esterification (DE) of pectin polysaccharides on the associative phase behaviour with a pea protein isolate.

Methods:

Pea protein was isolated from defatted yellow pea flour using an alkali extraction (pH 9) and isoelectric precipitation (pH 4.5) process. The formation of electrostatic complexes within mixtures of pea protein isolate (PPI) and various commercial pectin [a) citrus LM pectin (DE: 29%); b) sugar beet pectin (DE: 57%); c) apple pectin (DE: 79%); and d) citrus 85 pectin (DE: 86%)] were examined as a function of PPI: pectin mixing ratio (1:1, 2:1, 4:1, 8:1, 10:1, 15:1) and pH (8.0 – 1.5) by turbidimetric acid titration (at 600 nm) with a total biopolymer concentration of 0.05% (w/w). Homogeneous PPI and pectin solutions were used as controls. The critical pHs associated with complex formation (pH_c: associated with the formation of soluble complexes, $pH_{\phi 1}$: associated with the formation of insoluble complexes, pH_{opt}: associated with the pH where maximum coacervation occurs, $pH_{\phi 2}$: associated with the dissolution of formed complex) were identified using tangents on a graph of optical density vs. pH. The optimal mixing ratio was determined using the graph of maximum optical density vs. biopolymer mixing ratio. The relationship between the complex formation and charge neutralization was determined as a function of pH (7.0 -1.5) at optimal mixing ratios for each PPI: pectin mixture. Surface charge (or zeta potential) was measured using the electrophoretic mobility of the biopolymer mixtures using a zeta sizer.

Results:

Changes to the optical density vs. pH for homogenous pea protein isolate (PPI) solutions showed a bell curve turbidimetric profile as a function pH where, aggregation started at pH 6.5, reached a maximum near pH 3.8-4.8 and then broke up at pH 2.5, with maximum optical density occurring near 0.470. No optical spectrum was visible with the pectin polysaccharides. However, with the admixtures of PPI and pectin the turbidimetric spectrum changed considerably. For all mixtures, the bell shaped curve shifted to higher pHs and increased in magnitude relative to the homogenous PPI curve. For PPI: citrus LM (DE 29%) and PPI: citrus 85 (DE 86%) mixtures, the formation of soluble complexes occurred well above the isoelectric point (pI) of PPI (pH 4.8) at pH ~6.1 at the 1:1 mixing ratio, and then remained constant as the mixing ratio increased to 15:1. In contrast, the formation of soluble complexes involving mixtures of PPI: sugar beet (DE 57%) and PPI: apple (DE 79%) pectin

occurred slightly lower than the proteins pI value around pH 4.1-4.4 at the 1:1 mixing ratio, and then increased to pH ~ 6.1 at the mixing ratio of 2:1 where it then remained constant as the mixing ratio increased. Typically, above the pI of PPI both biopolymers should repel one another since they both carry a negative net charge. However, associative interactions are believed to initiate between the negatively charged pectin chains and positively charged patches on the protein's surface. However, large changes to optical density in all systems did not occur until the solution pH moved below the pI of PPI. The formation of insoluble complexes initiated between pH 3.5-3.8 for all systems at mixing ratios of 1:1, which then increased in a curvilinear manner as the mixing ratio increased to 15:1. The increase is thought to be associated with pectin interacting with PPI-PPI aggregates which would increase in size as more protein is present within the blend. The pH corresponding to the maximum optical density also followed a similar curvilinear trend, occurring at pH 3.3-3.5 at the 1:1 mixing ratio prior to increasing. The breakdown of electrostatic complexes for all systems occurred at pH 2 associated with charge neutralization on the pectin backbone, and remained constant regardless of the mixing ratio. The maximum optical density occurred at mixing ratios of 2:1 for blends of PPI with sugar beet, apple pectin and citrus 85 pectin, and then at a mixing ratio of 8:1 for blends of PPI with citrus LM pectin (Figure 1).

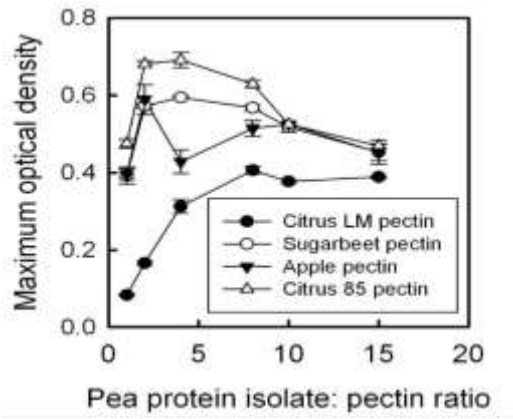

Figure 1. Maximum optical density obtained during a turbidimetric pH-titration of mixtures of pea protein isolate with various commercial pectin [a) citrus LM pectin (DE: 29%); b) sugar beet pectin (DE: 57%); c) apple pectin (DE: 79%); and d) citrus 85 pectin (DE: 86%)] as a function of mixing ratio. Data represent the mean ± one standard deviation (n = 3).

Surface charge (zeta potential) measurements of homogenous PPI solutions revealed the pI to be at pH 4.8, whereas the charge was negative and positive at pHs above and below the pI, respectively. All pectin chains were highly negatively charged over the complete pH range, approaching 0 mV close to pH 1.5. At pH 7, the charge for all pectin was ~-40 mV with the exception of citrus LM pectin which was ~-80 mV. The higher negative charge on that pectin resulted in the shift in the mixing ratio to 8:1, relative to the other systems. Admixtures of the PPI-pectin shifted where the charge equaled zero to below pH 4 for all systems.

Conclusion:

Findings from this study described the pH where associative phase separation occurs between PPI and various commercial pectin. The highly negatively charged citrus LM pectin resulted in much different association behaviours than the other pectin. Further exploration on the impact of protein functionality is currently ongoing.

Acknowledgements:

Financial support for this research was provided by the Natural Sciences and Engineering Research Council of Canada.

NO11

PEA PROTEIN FERMENTATION BY *LACTOBACILLUS PLANTARUM* FOR IMPROVED FUNCTIONALITY

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Pea proteins are gaining tremendous interest by the food industry as an alternative protein source to animal-derived proteins and soy due to their nutritional and functional attributes. However, in order to expand its market utilization ingredient producers should be exploring means of modifying its functionality and quality in order to tailor the pea protein ingredients to specific applications (e.g., beverages, baked goods or nutritional/breakfast snacks). Protein modification can occur by a variety of means, including thermal treatments (e.g., roasting, micronization or extrusion), enzymatic modification, physical shear and fermentation. Protein modification may involve inducing partial protein unfolding/disassociation or hydrolysis leading to the release of peptides and exposure of previously buried hydrophobic and hydrophilic amino acids. The change in surface properties can lead to improved functionality and digestibility.

Objective:

The overall objective of this research was to evaluate the effects of fermentation with *Lactobacillus plantarum* on the functional properties of pea protein concentrate (PPC).

Methods:

L. plantarum cells were cultivated until the late exponential phase of growth (~10 h) was reached and then used as the inoculum for fermentation. Fermentation experiments were carried out by inoculating *L. plantarum* pellets with a 25% (w/v) PPC solution (200 mL) at a concentration of 7 colony forming units (CFU) per g PPC at 32^{o}C for 11 h. Aliquots (60 mL) were taken at certain time points during fermentation, and then freeze-dried for 48 h using a freeze drier. Degrees of hydrolysis [%DH], surface characteristics [charge and hydrophobicity] and functional attributes [oil holding capacity (OHC), emulsification activity (EA) and stability (ES), foaming capacity (FC) and stability (FS), nitrogen solubility index (NSI) and water hydration capacity (WHC)] were determined [1-4].

Results:

Results showed that both crude protein and ash levels changed significantly during fermentation due to the increase in the bacterial biomass present. Protein levels increased sharply from ~43% to ~47% between 1 and 5 h of fermentation before leveling off which was hypothesized to be associated with the exponential growth of the *L. plantarum* cells. In contrast, the ash levels increased steadily from ~4% to 11% between 0 and 11 h of fermentation. Changes to the degree of hydrolysis of PPC during fermentation showed a sigmoidal increase where values increased from 0% DH at time 0 to a plateau at ~11% after 5 and 9 h, and then increased again to 13% after 11 h. Growth of *L. plantarum* led to the production of weak acids and release of small peptides from the proteins to cause a reduction in pH from 7.5 at time 0 to pH 4.3 after 11 h of fermentation.

Overall, the surface charge of the fermented PPC was found to be positive when adjusted to pH 4.0 and negative when adjusted to 7.0, since the proteins are below and above the isoelectric point of pea proteins (pI ~ 4.6), respectively. At pH 4.0, the charge increased from ~+14 mV at time 0 to a maximum of +27 mV after 1 h of fermentation, followed by a graduate decline to +10 mV after 11 h. In contrast, at pH 7.0, a gradual increase in charge at time 0 (-37 mV) to -27 mV after 11 h of fermentation was observed. At pH 4.0, hydrophobicity was found to be constant (~9 arbitrary units, a.u.) between 0 and 1 h of fermentation, and then increased to ~21 a.u. after 9 h, where it than plateaued. In contrast, at pH 7.0, hydrophobicity declined very slightly from ~8 a.u. at time 0 to ~7

a.u. after 11 h of fermentation). It is hypothesized that at pH 4.0, changes to the surface properties after 1 h of fermentation reflect hydrolysis of the PPC leading to a partial unraveling of the protein conformation and release of peptides which exposes buried reactive charged and hydrophobic sites.

Overall, oil holding capacity values for PPC increased from ~1.8 g/g at time 0 h to ~3.5 g/g after 1h, after which it fluctuated between 2.5 and 3.5 g/g. The emulsion forming abilities were found to be constant (emulsion activity ~45%) between 0 and 5 h of fermentation, then declined significantly to ~5-7% after 9 h of fermentation at pH 4. In contrast, emulsion formation was slightly less at pH 7 (emulsion activity ~35%) than at pH 4, however remained relatively constant over the 11 h of fermentation. The reduced emulsion forming properties at pH 7 may be the result of lower surface hydrophobicity of the protein structure, which remained low and constant. However, although better emulsions were formed at pH 4, they were inherently less stable than those formed at pH 7. At pH 4, emulsion stability was relatively constant at ~20% over the complete fermentation time, whereas at pH 7, emulsion stability increased from ~37% at time 0 to ~56% at 5 h, and then declined to ~20% after the 11 h fermentation period. The foaming capacity properties of fermented PPC increased from ~80% at time 0 to ~90% after 5 h of fermentation, and then declined to ~70% after 9 h of fermentation at pH 4. At pH 7 some variability was evident within the data, but foaming capacity remained relatively constant at ~70% most likely due to the relatively constant surface properties evident at this pH. Foam stability at pH 4 was relatively constant at ~20% up to 5 h of fermentation, and then dropped slightly to 9% after 9 h. In contrast at pH 7, foam stability remained relatively constant over the entire fermentation at ~20%. Overall, nitrogen solubility index remained relatively constant at ~8-11% regardless of the fermentation time at pH 4, whereas at pH 7, nitrogen solubility index decreased slightly from ~43% to 36% after 11 h of fermentation. For water hydration capacity, at both pHs, values declined from ~1-1.2 g/g at time 0 to 0.8-0.9 g/g after 5 h of fermentation, and then increased to 1.5-1.6 g/g after 9 h of fermentation

Conclusions:

Fermentation of pea protein concentrates by *L. plantarum* proved to be effective at altering both the surface and functional properties of the protein. The majority of changes occurred after 5 h of fermentation which was presumed to be due to the partial unraveling of the protein structure, release of peptides, and rise in the total protein content associated with the increase in microbial biomass. However more extensive fermentation studies are needed to start effectively tailoring the protein's functionality for better performance in specific applications (e.g., beverages, baked goods or nutritional/snack/breakfast foods).

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Acknowledgements:

Financial support for this research was kindly provided by the Saskatchewan Ministry of Agriculture, the Canada-Saskatchewan Growing Forward 2 Bi-lateral Agreement, and the Global Institute for Food Security (Saskatoon, SK). Special thanks are given to Parrheim Foods (Saskatoon, SK) for donating the pea protein concentrate used in this study.

NO12

STABILITY AND *IN VITRO* RELEASE BEHAVIOR OF ENCAPSULATED OMEGA FATTY ACIDS-RICH OILS IN LENTIL PROTEIN ISOLATE-BASED MICROCAPSULES

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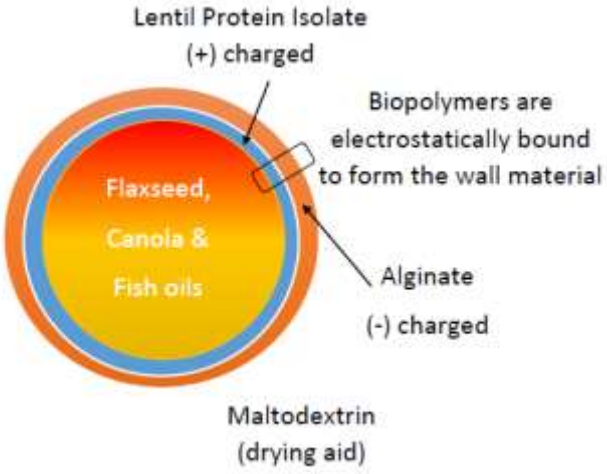
It is well known that omega fatty acids (e.g., omega-3, -6, and -9 fatty acids) play an essential role in human physiology, including the prevention and treatment of cardiovascular diseases and immune response disorders, development of the central nervous systems for infant growth, and maintenance of mental health (Shibasaki et al., 1999). However, due to their unsaturated nature, omega fatty acids-rich oils are chemically unstable and susceptible to oxidative deterioration and readily produce free radicals, which are deemed to negatively affect the shelf-life, sensory properties, and overall acceptability of the food products (Velasco et al., 2003). Therefore, microencapsulation of omega fatty acids-rich oils is considered as an effective way to protect those high value oils during processing and storage.

Objective:

The objective of this research was to encapsulate omega fatty acids-rich oils (e.g., canola, fish, and flaxseed oils) by spray drying using the combination of lentil protein isolate (LPI), sodium alginate and maltodextrin as the wall material, and to characterize the physical properties (e.g., moisture content, water activity, colour, wettability, particle size, surface oil, and entrapment efficiency), storage stability over a 30 d period and under accelerated storage conditions, and *in vitro* release behaviour of encapsulated oils under simulated gastrointestinal fluids.



Results:

All microcapsules displayed similar physical properties (e.g., moisture content of ~3.5%, water activity of ~0.35, particle size of ~8.9 μm, surface oil of ~2.4%, and entrapment efficiency of ~87.8%) regardless of the core material, except for the colour. Due to the visible differences in the colour of the oil itself, flaxseed oil capsules were more dark yellow in colour than fish oil capsules, followed by canola oil capsules.

Free fatty acids content (FFA), peroxide value (PV), and 2-thiobarbituric acid reactive substances (TBARS) were investigated to determine the storage stability (including hydrolytic stability and oxidative stability) of the encapsulated oils versus the free oils over 30 days storage period at room temperature. Overall, the encapsulated oils exhibited better storage stability than the free oils. The encapsulated canola oil showed better stability than the other encapsulated oils due to the refining step during the production of commercial canola oil (to remove most of free fatty acids) and lower degree of unsaturation. However, due to the presence of more double bonds on the fatty acids [e.g., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)], the encapsulated fish oil had a significantly higher increase in TBARS value than the encapsulated flaxseed oil during storage. In order to evaluate the antioxidative efficiency of microencapsulation, oxidative stability index (OSI)

was measured using a Rancimat for the encapsulated and free oils with/without antioxidants [e.g., butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ)]. Mostly, the oils with TBHQ had the maximum OSI, followed by the encapsulated oils and the oils with BHT, whereas the free oils had the minimum OSI. However, very interestingly, the encapsulated fish oil exhibited even higher OSI than the fish oil with TBHQ. Therefore, it is concluded that the combination of LPI, sodium alginate and maltodextrin provided the greatest protective effect to fish oils relative to the other combinations.

In vitro release characteristics of the encapsulated oils were studied under simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Only a minor amount of encapsulated oil (~3.2-8.9%) was released under SGF, with the majority (~62.6-73.4%) being released after sequential exposure to SIF, which was due to the greater degradation of microcapsules under the longer digestive process. Greater release was seen for polyunsaturated fatty acids relative to saturated and monounsaturated fatty acids from the microcapsules under the exposure of simulated gastrointestinal conditions (SGF + SIF), because fatty acids with a longer chain length and more double bonds are hardto stay at the surface of microcapsules and need a longer time to be released (Pourashouri et al., 2014).

Conclusion:

In conclusion, microcapsules prepared with LPI, sodium alginate and maltodextrin are appropriate to be used as a universal platform to encapsulate high value omega fatty acids-rich oils to be applied in commercial food and supplementary products.

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Acknowledgements:

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NO13

THE EFFECT OF DIFFERENT PROCESSING METHODS ON THE PROTEIN EFFICIENCY RATIO OF RED AND GREEN LENTILS, GREEN AND YELLOW SPLIT PEAS AND CHICKPEAS.

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Background

Protein quality is an indication of the protein content and the availability of that protein for metabolic use. Although there are multiple ways in which to measure protein quality, the Protein Efficiency Ratio (PER) is the method currently required by Health Canada. In this method rodents are fed a diet comprised of 10% protein and their weight gain over a four week period is compared to a control group which is consuming a casein diet, also comprised of 10% protein. The protein efficiency ratio is then calculated by dividing the overall weight gain by total protein consumed. The two primary factors that can alter the inherent quality of a protein source are the amino acid composition and the availability of those amino acids for absorption, which can vary depending on the presence of anti-nutritive factors.

Pulses are high in protein/fiber and generally low in fat. Much like other plant-based protein sources pulses are limiting in one of more amino acids when compared to the requirements for a growing human, typically either tryptophan or the sulfur amino acids (cysteine and methionine). Pulses crops are rarely consumed raw, due to palatability and the presence of anti-nutritive factors, and different processing methods could result in different PER values for the same crop. In this study we investigated the PER of pulse crops, specifically lentils (red and green), peas (green and yellow) and chickpeas after being processed through either baking, cooking/boiling or extrusion. The purpose of this study was to directly investigate the effect of these processing methods on the protein efficiency ratio of peas, lentils and chickpeas.

METHODS

Baked samples were prepared by mixing flour with water, the resulting mixture being sheeted and baked at approximately 380 °C and milled to flour (Food Development Centre). Cooked samples were soaked overnight, rinsed (pea/chickpea) and boiled until done, freeze dried and milled to flour (University of Manitoba). Extruded samples were passed through a Clextral Evolum HT 25 twin screw extruder to produce a puff which was subsequently milled to flour. The resulting flours were analyzed via LECO combustion to determine nitrogen content, which was multiplied by a factor of 6.25 to generate crude protein content, with fat content being determined by gravimetrics (Table 1). Diets contained all nutritional requirements for the growing rat and differed only by the protein source, which comprised 10% of the diet.

Diet consumption was monitored daily, with bodyweight measurements being taken weekly. At the end of the trial (28 days after initiation of test diet) PER was calculated as weight gain (g)/weight of protein consumed (g). Data was analyzed via Two-Way ANOVA with Tukey's MC test.

RESULTS AND DISCUSSION

Processing did not alter the crude protein content for any of the pulses investigated (Table 1). The dry matter tended to be highest in the cooked samples, followed by baked and then extruded. In all cases the baked samples had a higher fat content than either the baked or cooked pulses. Extrusion resulted in a higher PER value than baking for green lentil, red lentil and chickpea, with cooked pulses have a higher PER value than baked for red lentil, yellow split pea and chickpea. Interestingly, processing had no effect on the PER value for green split pea. These data

indicate that preparatory method does alter the PER of certain pulse classes, potentially due to alteration of amino acid content or protein digestibility.

Table 1: Proximate analysis and Protein Efficiency Ratio of Processed pulses

Ingredient	% CP	%DM	% CF	PER
Baked Green Lentil	25.44	97.32	2.21	1.09
Cooked Green Lentil	25.67	99.47	2.06	1.21
Extruded Green Lentil	24.65	95.13	1.48	1.34
Baked Red Lentil	25.93	97.49	2.34	0.98
Cooked Red Lentil	26.62	99.57	1.62	1.41
Extruded Red Lentil	26.86	95.41	1.08	1.30
Baked Green Split Pea	23.91	97.81	2.27	1.84
Cooked Green Split Pea	23.84	99.7	1.3	1.78
Extruded Green Split Pea	23.99	95.64	0.32	1.89
Baked Yellow Split Pea	22.37	95.81	2.84	1.85
Cooked Yellow Split Pea	22.29	97.44	1.46	2.08
Extruded Yellow Split Pea	23.48	95.62	1.37	1.81
Baked Chickpea	20.19	96.67	9.58	2.85
Cooked Chickpea	21.59	97.68	7.59	3.06
Extruded Chickpea	20.21	95.57	6.88	3.18

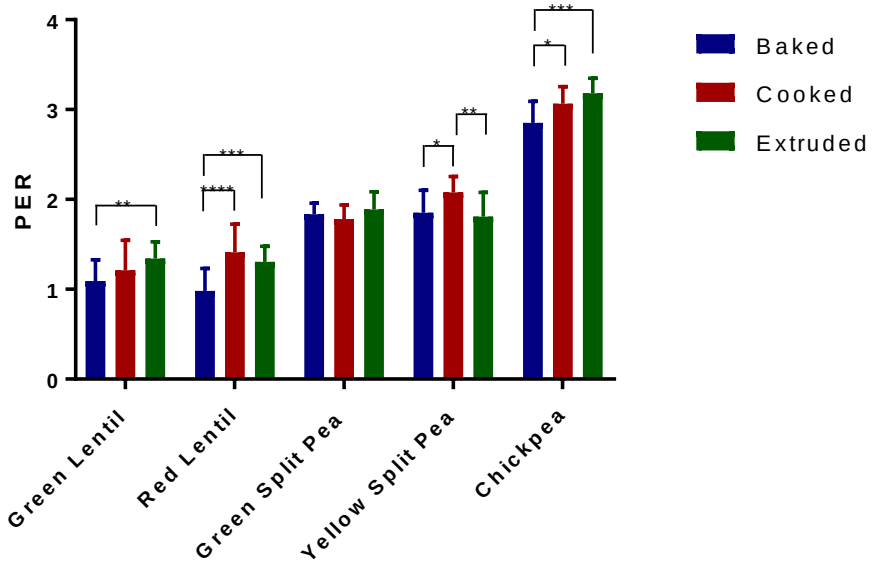

Figure 1: Protein Efficiency Ratio (PER) of processed pulses. Data was analyzed via Two-Way ANOVA with Tukey's MC test. Mean ±SD . Bars with different letters are significantly different, * = p<0.05, **= p<0.01 *** = p<0.001, **** = p<0.0001.

NO14

IRON FORTIFICATION IN CHICKPEA: A POSSIBLE SOLUTION FOR IRON DEFICIENCY IN HUMANS

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Iron deficiency (ID) is the most common nutritional disorder among all micronutrient deficiency due to insufficient absorbable iron. Chickpea (*Cicer arietinum*) is a potential vehicle for fortification as it contains high protein with Fe. Iron fortification in chickpeas by using iron fortificants to augment absorbable iron may address iron deficiency in less developed countries. Different Fe fortificants were used by simple spraying and heating method to improve the bioavailable Fe in chickpea seeds and flour. Sensory acceptability of fortified chickpea products was evaluated by regular chickpea consumers. A lab based test was done to check the bioavailable Fe by human cells. Experiments with fortified desi and kabuli chickpea products showed that NaFeEDTA and FeSO4.7H2O were effective for increasing Fe concentration. A 9point hedonic scale of sensory evaluation of appearance, colour (raw and cooked) and taste (cooked) showed that Fe-fortified chickpea dal and chapati were acceptable to consumers. *In vitro* assessment of bioavailability of fortified chickpea increased bioavailable Fe with all three fortificants. These results link the improved Fe content in chickpea seeds and flour associated with increased bioavailable Fe. Fortified chickpea products can cover a major part of recommended daily Fe requirement in a very cost-effective way. Application of Fe fortification technique can be a potential solution in regions where Fe deficiency and chickpea consumers coexist.

NO15

MAPPING OF A PEPSIN-RESISTANT PEPTIDE AND IDENTIFICATION OF *O*-GLYCOSYLATION SITES IN THE α SUBUNIT OF THE 11S GLOBULIN LEGUMIN FROM COMMON BEAN

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Unlike in soybean and other legumes, 11S globulins are relatively minor constituents of storage proteins in common bean (*Phaseolus vulgaris*). However, it was previously reported that the 11S globulin, legumin, contains a peptide of ca. 20 kDa which is resistant to simulated gastrointestinal digestion. In grain protein, resistance to proteolytic digestion often correlates with allergenicity. An approach combining purification and mass spectrometry was used for biochemical characterization. Using purified legumin, the peptide of ca. 20 kDa was found to be resistant to pepsin digestion in a pH-dependent manner and was mapped to an internal fragment of the α-subunit. The same fragment coincides with a peptide resistant to chymostrypsin digestion. The α-subunit of legumin exhibits a reduced mobility in SDS-PAGE, by a factor of ca. 10 kDa, and contains a consensus motif for plant *O*-glycosylation. Using a peanut agglutinin purified legumin, five contiguous sites of *O*-glycosylation were identified by liquid chromatography and tandem mass spectrometry (LC-MS/MS) after trypsin digestion. Sites of *O*-glycosylation were identified as hydroxyproline residues substituted with one or two galactoses following fragmentation. For the site corresponding to the consensus motif, the mass of the *O*-glycosylated peptide was greater than the mass of the non-glycosylated version by ca. 3 kDa. In LC-MS/MS, this peptide co-eluted with a hexose chain of at least 10 residues. To our knowledge this represents the first detailed report of an *O*-glycosylated seed storage protein.

NO16

ANTIOXIDANT ACTIVITY OF WATER AND AQUEOUS ETHANOL EXTRACTS OF LENTIL (*Lens culinaris* L.) SEED COAT

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Lentil seed contains phenolic compounds with potent antioxidant activity which are concentrated in the seed coat rather than in cotyledon[1]. The seed coat fraction is a byproduct of commercial processing of lentil, especially with red lentil, and would be an economical source for recovery of phenolic compounds. The extracts of seed coat may provide a concentrated form of antioxidants with higher antioxidant efficacy than the unfractionated seed coat material containing mixed and non-active compounds. In addition, the differences in the polarity of the phenolic compounds may influence their antioxidant activity in aqueous, lipid or aqueous-lipid food systems.

Water and 70% (v/v) aqueous ethanol extracts of the seed coat of two cultivars (CDC Greenland, a large green and CDC Maxim, a small red variety) of lentil were analyzed for their antioxidant capacity in the form of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)[2] and DPPH (2,2-diphenyl-1-picrylhydrazyl)[3] stable free radical scavenging activity, Fe^{2+} chelating activity[3] and inhibition of iron induced phospholipid (PL) peroxidation[4] at different concentrations of total phenolics (100-600 ppm GAE equivalents).

The seed coat represented 7.38 ± 1.68 and 7.01 ± 1.04 (%) of the total seed weight of CDC Greenland and CDC Maxim respectively. Seed coats of both cultivars of lentil contained similar amounts of moisture, ash, protein and fat (9%, 3%, 8% and 0.3% respectively). The total extractable phenolic content (TPC) of seed coat in 70% ethanol and water ranged from 47.37 ± 4.57 to 48.19 ± 1.94 and 41.92 ± 2.32 to 45.28 ± 1.75 mg/g of seed coat, respectively and there was no significant difference between the two cultivars with respect to the total extractable phenolic content in each solvent. The seed coat extracts exhibited a concentration dependent antioxidant activity irrespective of the cultivar and extraction solvent except for the inhibition of PL peroxidation. The DPPH and ABTS free radical scavenging activity and the Fe^{2+} chelating activity of seed coat extracts increased with increasing concentration of phenolic content up to 400 ppm and plateaued after. At TPC below 300 ppm, the aqueous ethanol extracts showed greater ABTS free radical scavenging ability whereas water extract had greater DPPH free radical scavenging activity. The aqueous ethanol extract of CDC Greenland showed greater Fe^{2+} chelating activity at 300 ppm concentration than that of other extracts. The antioxidant effect of seed coat extracts against the peroxidation of PL did not show a concentration dependent change in the activity and there was no significant difference in the inhibition of PL peroxidation between these cultivars of lentil. However the ethanolic extract of the seed coat of both cultivars showed a greater inhibition ability of PL oxidation than that of water extracts.

When compared with four other food grade antioxidant compounds; (+/-) - α- tocopherol, (+)-catechin, sodium ascorbate and Herbalox® at 300 ppm concentration (Figure 1), the extracts of seed coat showed a higher free radical scavenging activity than that of Herbalox® and sodium ascorbate and comparable activity with (+)-catechin and tocopherol. The ethanolic extract of CDC Greenland seed coat had the greatest ability to chelate Fe^{2+} among the antioxidants studied. The ability to inhibit PL peroxidation of aqueous ethanol extract of CDC Greenland seed coat was comparable to (+)-catechin and (+/-) - α- tocopherol and superior to Herbalox® and sodium ascorbate. The PL inhibition activity of other seed coat extracts was similar to the activity of Herbalox® and sodium ascorbate.

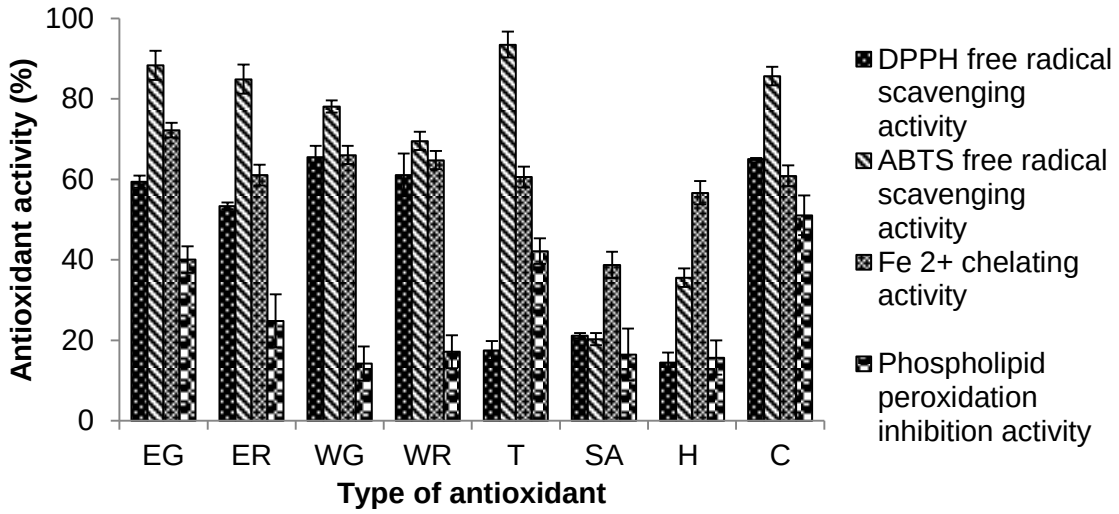

Figure 1. Antioxidant activity of seed coat extracts and other food-grade antioxidants at 300 ppm concentration of total phenolics (EG: ethanol extract of CDC Greenland, ER: ethanol extract of CDC Maxim, WG: water extract of CDC Greenland, WR: water extract of CDC Maxim, T: (+/-) - α- tocopherol, SA: sodium ascorbate, H: Herbalox®, C:(+)-catechin).

The results of the current study revealed that water or 70% (v/v) ethanol can extract phenolic compounds of lentil seed coat. These components have significant antioxidant activity that depends on the cultivar, concentration of phenolics and composition of extraction solvent. However further studies are needed to understand their actual antioxidant activity in complex food systems such as meat.

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NO17

BLACK BEANS REDUCE HYPERTENSION-RELATED VASCULAR REMODELLING: A DIETARY INTERVENTION STUDY IN SPONTANEOUSLY HYPERTENSIVE RATS

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More than 1 in 5 Canadian adults have hypertension (1). Compared to normotensive Canadian adults, the mortality rates of hypertensive individuals are 60% higher because they are more likely to develop dementia, kidney disorders, and cardiovascular disease (1). Furthermore, the etiology of hypertension is unknown in 95% of cases making treatment difficult (2). The goal of prescription drug therapy is to control high blood pressure, which is treating the symptom rather than the underlying cause of hypertension. Moreover, 30% of individuals with hypertension have uncontrolled high blood pressure, indicating that for some individuals, medications are unable to provide symptomatic relief (1). Thus, it is apparent that new methods to reduce hypertension among Canadians are needed. Dietary strategies to reduce hypertension have been previously explored. Foods that provide fibre, potassium, and plant protein, and that are low in saturated fat and sodium have been shown to reduce blood pressure (3). In addition, phenolic compounds from foods have been shown to benefit the vasculature and exert hypotensive effects (4). Pulses are grown in abundance in Canada and comprise all the aforementioned dietary characteristics. A review and meta-analysis found that a mean intake of 162 g/day of cooked pulses significantly lowered blood pressure among subjects who were either prehypertensive or normotensive (5). In spontaneously hypertensive rats (SHR), the consumption of lentils for 4 weeks resulted in significantly lower blood pressure compared to the control (pulse-free) fed SHR (6). Furthermore, the lentil diet was able to mitigate vascular remodelling. In hypertension, the vessel wall can thicken (remodel), which exacerbates high blood pressure (7). After the 4-week intervention, the blood vessel media:lumen ratio of the lentil fed SHR was not significantly different from normotensive Wistar Kytoto (WKY) rats (6). However, this ratio was significantly increased in the control fed SHR compared to WKY (6). Currently there are no treatments that address this underlying physiological change to the blood vessels of hypertensive individuals. While these aforementioned results were not found with a bean diet, it is relevant to point out that the researchers had examined a mixture of beans, including black, navy, red kidney, and pinto beans. When comparing bean types, black beans tend to have a greater total phenolic content, and thus, black beans may be more effective than other bean types for alleviating hypertension and should be examined separately (8).

We hypothesized that black beans would be able to mitigate hypertension in SHR by attenuating rises in blood pressure and vascular remodelling. Our objectives were to evaluate blood pressure and blood vessel structure in SHR fed a black bean diet for 8 weeks, comparing to control (pulse-free) fed SHR and normotensive WKY rats. Black beans (~90% Eclipse and ~10% unknown variety) were obtained from Legumex Walker (Plum Coulee, MB, Canada), and were soaked overnight, cooked for 1 hour, then freeze-dried and ground to a powder. The black bean powder was added to the diet at 30% w/w. The background diet for the black bean and the pulse-free control diets was the American Institute of Nutrition 93 Growth diet for rats. The animals were ordered from Charles River Laboratories (St. Constant, QC, Canada) and were housed in the R.O. Burrell Laboratory animal facility at the St. Boniface Hospital Albrechtsen Research Centre (Winnipeg, MB,

Canada). When the rats were 16 weeks of age, baseline measurements of blood pressure were recorded by the tail-cuff method and the SHR were randomized to receive either the black bean or control diet, and the WKY were given the control diet (n=10/group). The diets were consumed for 8 weeks, after which time final blood pressure measurements were recorded, the animals were sacrificed, and the aortae were harvested for histological examination. Specifically, a 1 cm portion of aorta was embedded in Optimal Cutting Temperature compound, frozen and sliced with a Cryotome to obtain cross-sections that were fixed to glass microscope slides. Staining of the cross-sections was done with an Elastin Stain Kit (Sigma-Aldrich; St. Louis, MO, USA), and the vessel structure was assessed using ImageJ software (9). All data were analyzed by ANOVA and the Duncan's Multiple Range test using SAS (version 9.3). Results were considered significant at $p<0.05$.

Specific results of this study will be made available at the time of publication. However, the results suggest that the consumption of black beans may attenuate hypertension-related vascular remodelling, an underlining contributor to high blood pressure.

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NP18

COMPARISON OF PHYTOCHEMICALS AND ANTIOXIDANT CAPACITY IN THREE BEAN VARIETIES GROWN IN CENTRAL MALAWI

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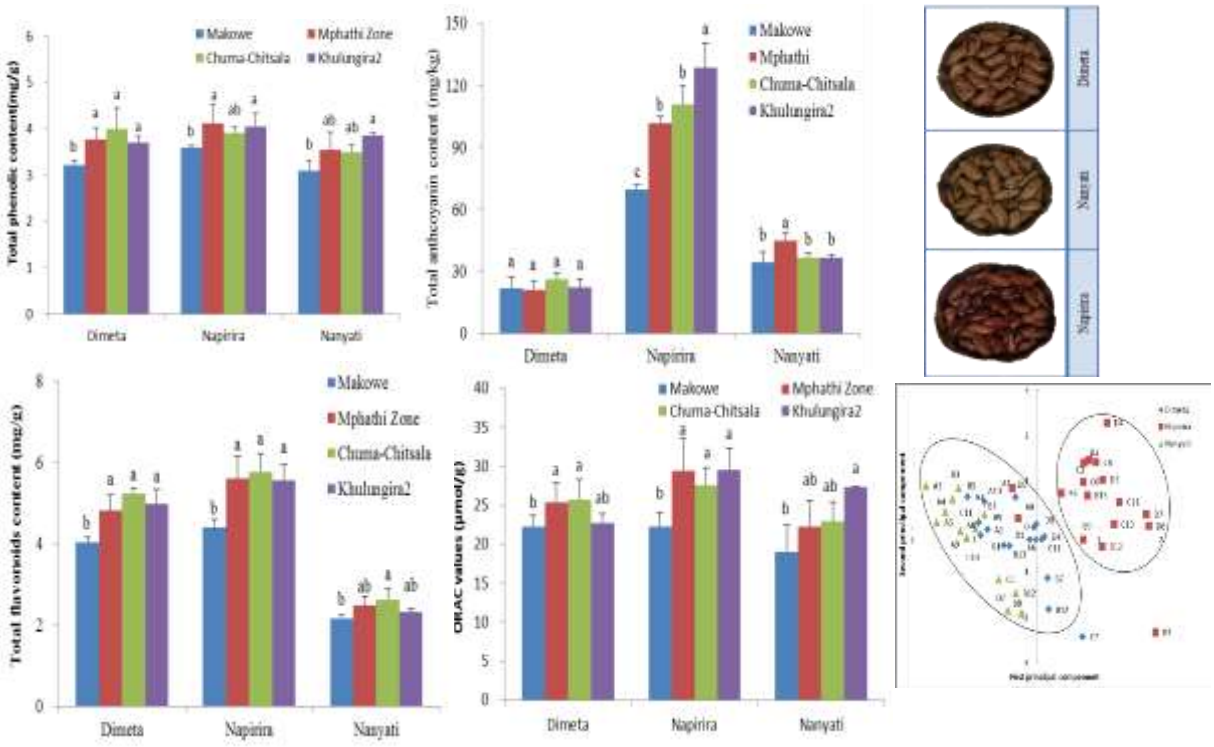
Abstract
This study was completed as part of a broader project on agroecological methods to improve food security and nutrition for smallholder farm households. Statistical analysis showed significant differences ($p<0.05$) in common beans (*Phaseolus vulgaris* L.) in their total phenolic acids, total anthocyanins, total flavonoids, as well as the oxygen radical absorbance capacity among the different sampled villages. PCA indicated that total phenolic acids, total anthocyanins, total flavonoids, total carotenoids and ORAC could serve as parameters to establish a bean classification according to the geographical area of production.
Key words: Bean varieties; Phytochemicals; Antioxidant capacity; Principal component analysis; Hierarchical cluster analysis

Introduction
Common beans (*Phaseolus vulgaris* L.) are a good source of phenolics and other phytochemicals. Beans are an important crop for nutrition and the Malawi Government maintains an active research and development program for improvement of the crop. Moreover, ecological conditions vary greatly in Malawi and the bean-growing regions are very scattered through its territory. In addition, the wide variety of microclimates around the country, leads to bean production with high variability in its chemical composition.
The aims of the current work were: (1) to study the influence of variety and geographical production area on the total phenolic, total anthocyanin, total flavonoid, total carotenoid content and antioxidant activity in bean varieties from different growing areas in central Malawi, and (2) to evaluate the possibility of establishing a classification based on the geographical areas of the growing regions.

Material and Methods
A total of forty-seven bean samples (Phaseolus vulgaris L.) (Dimeta, Nanyati, Napirira varieties) were collected from Makowe, Mphathi, Chuma-Chitsala and Khulungira2 zones in central Malawi in 2014.
The dried beans were subsequently milled into powder with an electric mill (Bel-Art Products, Pequannock, NJ, USA), sieved (40 mesh) and stored at 4^{o}C prior to extraction.

Results and Discussion

Significant differences (P<0.05) in total phenolic content (TPC) (2.92-4.97 mg/g), total anthocyanin content (TAC) (14.52-152.31 µg/g), total flavonoid content (TFC) (2.01-6.38 mg/g) and oxygen radical absorbance capacity (ORAC) (16.75-24.51 µmol/g) were found among the different sampled villages, showing a significant effect of the producing region on these parameters. Napirira bean had the highest TAC and TFC among the three bean varieties. The beans in Makowe Zone had lower polyphenols than in other locations. Results of principal component analysis (PCA) and hierarchical cluster analysis (HCA) indicate that phytochemicals and antioxidant capacity could serve as parameters to establish a bean classification according to the geographical area of production.



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NP 19

THE IMPACT OF A DRY THERMAL TREATMENT ON THE FUNCTIONALITY AND IN *VITRO* PROTEIN DIGESTIBILITY TREATED BARLEY-PULSE FLOURS

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Issues surrounding food insecurity stretches around the globe, as countries search for sustainable high quality protein sources to support the growing population, which is expected to reach over 9 billion by 2050. Pulses are recognized as being a rich source of proteins and other micro/macronutrients that help fill the protein gap; however they tend to be deficient in the thiol amino acids (cysteine + methionine), but rich in lysine. And as such, they are often consumed alongside cereal grains especially in the developing world as a complementary protein source. Cereals tend to be deficient in lysine but rich in the thiol containing amino acids.

Objective:

The overall objective of this research was examine the functional properties and in vitro protein digestibility (IVPD) of flour blends derived from hull less barley with a variety of pulses in the absence and presence of a dry heat treatment.

Methods:

Whole seeds of desi chickpea, red lentil, green pea, faba bean and hull less barley were ground into coarse flour using a disc mill and then into finer flour using a UDY Cyclone Sample Mill to pass through a 500 µm sieve. Pulse fours were thoroughly mixed with hull less barley flour at ratios of 50:50, 60:40 and 70:30 to form blended flours. The blended and pure flours were subjected to dry heat at 121°C for 25 min (Cardoso et al. 2014). Oil holding, water hydration and foaming properties for all the flours were determined according to Stone et al (2015). IVPD was determined using a multi-enzyme system comprised of chymotrypsin from bovine pancreas, trypsin from porcine pancreas, and protease from *Streptomyces griseus* to digest the protein solution at pH 8 for 10 min (Hsu et al. 1977).

Results:

Oil holding capacity for untreated hull less barley flour and pulse flours were found to be ~1.3 g/g and 1.0-1.1 g/g, respectively. The addition of dry heat or blending (regardless of the ratio) did not significantly alter the oil holding capacity data. Water hydration capacity (WHC) for untreated hull less barley was 1.3 g/g, whereas values for pulse flours ranged between 1.2-1.8 g/g. With chickpea flour being the lowest, followed by green pea and faba bean flour which were similar, and then red lentil flour displaying the greatest WHC. Blending in the barley had little effect on the WHC properties, regardless of the ratio. However the addition of dry heat, overall led to improved WHC relative to untreated flours, with the exception of the pure chickpea and faba bean flours. Foaming capacity for untreated hull less barley and pulse flours were 104% and 156-200%, respectively. Of the pulse flours, faba bean was found to have the best foam forming properties, whereas red lentil flour was the worst. The addition of dry heat resulted in a decrease in foaming capacity for chickpea and faba bean flours, an improvement for barley, and had no effect on red lentil and green pea flours. In the case of the blended flours, foaming capacity was similar for the barley-pulse blends involving faba bean and red lentil flours, however varied when mixed with green pea flour, and declined with chickpea flour. Foam stability of untreated hull less barley and pulse flours was ~18% and 56-78%, respectively. In the case of the pulse flours, foam stability was highest for chickpea and green pea, and lowest for red lentil. The addition of dry heat led to an increase in foam stability for

barley-faba bean blends, but decreased for barley blends with chickpea, green pea or red lentil flours.

In vitro protein digestibility (IVPD) of untreated hull less barley and pulse flours were ~79% and 77-86%, respectively (Table 1). Of the pulses, the digestibility was greatest for green pea and lowest for chickpea flour. Blending did not seem to impact the IVPD significantly. Overall, the addition of dry heat resulted in a loss of IVPD by ~4-5% irrespective of the flour or flour blend.

Table 1. *In vitro* protein digestibility (IVPD) of raw and treated flours with dry heat. Data represent the mean ± one standard deviation (n=3).

Sample	Pulse: Barley ratio	IVPD Raw flours	IVPD Flours treated with dry heat
a) Pulse: Barley blends			
Desi Chickpea	50:50	79.0 ± 1.8	75.1 ± 0.5
	60:40	81.6 ± 2.2	75.6 ± 1.3
	70:30	80.1 ± 1.3	75.9 ± 2.2
Red lentil	50:50	78.3 ± 0.3	69.7 ± 2.3
	60:40	82.0 ± 0.1	72.8 ± 1.7
	70:30	85.8 ± 1.1	71.7 ± 0.6
Faba bean	50:50	82.9 ± 2.4	75.3 ± 1.1
	60:40	84.5 ± 0.4	77.8 ± 0.3
	70:30	83.4 ± 0.3	81.0 ± 5.1
Green pea	50:50	85.0 ± 0.1	71.9 ± 2.0
	60:40	83.4 ± 1.7	71.1 ± 0.1
	70:30	86.2 ± 0.3	77.0 ± 2.0
b) Flours alone			
Hull less barley	0:100	79.4 ± 0.6	75.9 ± 2.1
Desi chickpea	100:0	77.5 ± 0.3	68.4 ± 2.6
Red lentil	100:0	81.0 ± 2.0	80.6 ± 3.7
Green pea	100:0	86.3 ± 1.1	77.6 ± 2.2
Faba bean	100:0	83.1 ± 0.8	78.8 ± 5.0

Conclusions:

In summary, the impact of blending and dry heat on the functional properties was variable and highly dependent on the ratio and pulse present within the barley blend. IVPD data was most affected by the addition of dry heat, where a loss in digestibility was observed.

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Acknowledgements:

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NP 20

EFFECT OF PROCESSING ON LECTINS IN PULSES AVAILABLE IN CANADA

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Abstract:
The effects of pulse type and soaking, cooking, roasting and germination on the level of lectins (hemagglutinins) in a range of pulses available in Canada were investigated, using soybean as a control. Lectins were evaluated using a looking for evidence of agglutination as a function of concentration. Lectins levels varied with pulse type; the highest values were obtained for soybean. In raw pulses lectin levels were highest in beans and lowest in chickpeas. Soaking had no effect on lectin levels. Roasting reduced lectin levels in some pulses and cooking (boiling) eliminated all lectin activity. Germination effectively reduced lectin activity in soybeans, chickpeas and navy beans, but was not effective with the other pulse crops. The method for evaluating lectins presented some challenges as it was based on a subjective evaluation at incremental dilutions and a difference of one dilution level could double the determined value. Hemagglutinins are present in all pulse crops but to varying degrees; cooking at a high moisture level can effectively remove these antinutritional factors.

Introduction:
Legumes are important for human beings and domestic animals around the world, particularly in tropical and subtropical countries. Pulses, also called grain legumes, are dry edible seeds harvested from leguminous plants. Underutilization of pulses is often attributed to the presence antinutritional factors including the lectins or hemagglutinins. When evaluating pulse protein quality, these antinutritional factors should not be overlooked. Lectins are widely distributed in plant material and are known for their ability to bind reversibly to specific glycoproteins and carbohydrates, including those on cell membrane. This can result in growth inhibition in animals, decreased nutrient absorption, (Campos-Vega et al., 2010). They can also influence metabolism as well as the immune system and can lead to damage in body tissue (van der Poel, 1990). To improve pulse utilization, numerous studies have been conducted on reduction or elimination of lectins using different food processing methods and treatments. The present research was undertaken to provide an evaluation of lectins and phytic acid in a range of market classes of pulses available in Canada. To reduce the level of these antinutritional compounds, a range of processes, including soaking, cooking, roasting and germinating, were examined.

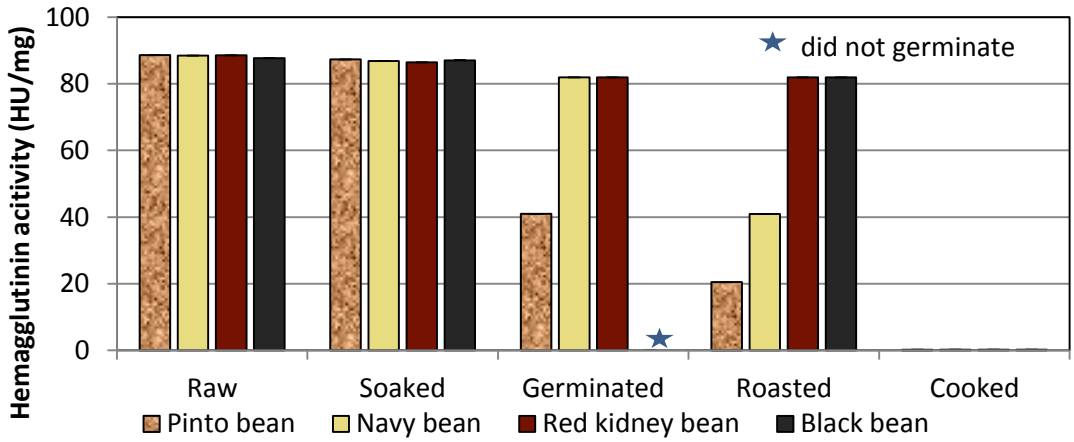
Materials and Methods:
Pulses examined included green and yellow peas, red, Spanish brown, French green, medium green and large green lentils, fababean, Desi and Kabuli chickpeas, and pinto, dark red kidney, navy and black beans. Soybeans were included for comparison. Four processes were evaluated. Soaking involved immersing seeds in water at a 1:5 seed to water ratio at room temperature for 4 hours. To cook, soaked seeds were heated at 95°C for 1 h at a 1:5 seed to water ratio. For roasting, seeds were tempered to 12% moisture and roasted at 100°C for 1 h and to germinate, seeds were placed in an Automated Sprouter for 3 days at room temperature. Lectins were extracted in 0.9% NaCl (1 g in 10 mL) and serially diluted (1 to 8191 times) in a 96 well plate. An equal volume of 2% rabbit red blood cells was added to each well. Wells were examined in a microscope and the dilution at which at least 5 cells had aggregated was the dilution used in the calculation. Hemagglutinin activity (HU/mg) was calculated as:

$$\mathrm{HU/mg} = \frac{\text{Dilution} \times \text{mL extract/mg flour}}{\text{Volume in well}}$$

Results and discussion:
The levels of lectins in pulses available in Canada varied widely. The lectin contents in all the pulses were lower than in soybean. Of the pulses investigated, lectin levels were highest in beans (~85HU/mg) followed by lentils, peas, fababean with the lowest values (~2.5HU/mg) in chickpeas.

Both legume type and processing treatment affected the level of lectins. Soaking had no impact on lectins. Roasting decreased lectins for soybean, fababean, chickpeas, red lentils, pinto beans and navy beans (Figure 1); for peas, most lentils, kidney beans and black beans, lectins were unaffected by roasting. Cooking of presoaked seeds was the most effective way to remove lectins in all seed (except fababean) with reductions of 80 –100%; almost complete elimination was achieved for bean (Fig. 1). Germination, as expected, reduced lectins in soybeans; however, with pulses, reductions were only seen in the chickpeas and pinto beans.

Figure 1. Effect of processing on lectins in beans



Conclusions:
Information obtained from the present study demonstrates that lectins can present a problem in some pulse crops but the levels can be controlled with proper processing. Moist heat was effective for all crops, while roasting and germination were effective only for selected pulses.

Acknowledgements:
Funding for this project was provided by Saskatchewan Pulse Growers (Agriculture and Agri-Food Canada Growing Forward 2 program) and University of Manitoba Graduate Fellowship.

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NP21

VARIATION IN DIETARY FIBRE AND OLIGOSACCHARIDE CONTENTS OF DIFFERENT BEANS (*Phaseolus vulgaris*) GROWN IN MANITOBA

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This study was to investigate how cultivar and growing location affect dietary fiber and oligosaccharide contents of beans grown in Manitoba. Twenty bean cultivars were grown in three replications in a randomized complete block design at two locations in Manitoba (Morden and Portage la Prairie) in 2013. The cultivars chosen are commonly grown in Canada and frequently used in crossing in the Morden Dry Bean Breeding Program in Manitoba, Canada. Insoluble (IDF), soluble (SDF) and total dietary fiber (TDF), moisture and oligosaccharide contents were determined according to the methods.[1-3] Statistical analyses were conducted using the SAS (v.9.4, SAS Institute, Cary, NC). The analysis of variance (ANOVA) for the main effects (cultivar and growing location) and interactions were determined using the GLM procedure. The Duncan multiple range test was used to separate means and significance was accepted at $p<0.05$.

Analysis of variance showed that cultivar and growing location had a significant effect on IDF, TDF, stachyose and verbascose content (Table 1). SDF and raffinose content were only affected by cultivar. Interaction of cultivar-by-location significantly affected SDF. IDF of different beans ranged from 153.5-198.8 (g/kg dry matter), SDF from 22.7 to 46.4 (g/kg dry matter) and TDF from 181.5 to 242.6 (g/kg dry matter), respectively. Raffinose varied from 2.0 to 9.4 (g/kg dry matter), stachyose from 29.5 to 42.9 (g/kg dry matter) and verbascose from 0.34 to 2.69 (g/kg dry matter), respectively.

Table 1 Analysis of variance of the effect of cultivar and location on insoluble (IDF), soluble (SDF), total (TDF) dietary fibre and oligosaccharides content of dry beans

Composition	Cultivar (C)	Location (L)	C x L
Dietary fibre (g/kg dry matter)			
IDF	***	***	ns
SDF	***	ns	*
TDF	***	***	ns
Oligosaccharides (g/kg dry matter)			
Raffinose	***	ns	ns
Stachyose	***	**	ns
Verbascose	***	*	ns

***,**, *=significant at P<0.001, P<0.01, and P<0.05, respectively.
ns= no significance.

Beans grown in Morden had a lower average IDF (175.7 g/kg dry matter) and TDF content (212.5 g/kg dry matter) than in Portage la Prairie (182.5 and 218.2 g/kg dry matter, respectively) whereas beans from Morden had a higher average SDF (36.8 g/kg dry matter) than in Portage la Prairie (35.4 g/kg dry matter).

Beans grown in Morden had a higher average raffinose (5.0 g/kg dry matter) and verbascose content (0.94 g/kg dry matter) than in Portage la Prairie (4.8 and 0.82 g/kg dry matter, respectively) whereas beans from Morden had lower average stachyose content (36.9 g/kg dry matter) than in Portage la Prairie (37.6 g/kg dry matter).

Results obtained in this study showed that the contents of dietary fiber and oligosaccharides in beans were largely affected by cultivar and growing location. These findings indicated that selection of a bean cultivar with high dietary fibre and reduced levels of oligosaccharides should take into consideration, not only the genetic factors, but also the growing locations during the growing season. This information is also useful to pulse marketers, nutritionists and functional food manufacturers, who wish to utilize and promote the high fibre content of this crop.

Acknowledgements

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NP22

EFFECTS OF PROCESSING ON CHYMOTRYPSIN ACTIVITY IN CANADIAN PULSES

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Abstract: A variety of raw and processed pulses including peas, beans, lentils, fababean and chickpeas were evaluated for their chymotrypsin inhibitor activity through enzymatic analysis. Soybean was included for comparison. Simple processing techniques such as germinating, roasting and boiling were evaluated. Chymotrypsin activity was determined using casein as the substrate; breakdown products with and without the presence of the inhibitor were measured and chymotrypsin inhibitory units (CIU) calculated. Levels of chymotrypsin inhibitors varied widely between raw samples with the lowest value for fababean (~1 CIU/mg) to a high of 30 CIU/mg for soybean. In general, all processing treatment reduced the level of CIU. Roasting reduced the CIUs in pulses by 45 to 90% with lower reduction in fababean, peas and lentils than in beans and chickpeas. A 97% reduction was seen for soybean. Boiling completely destroyed the chymotrypsin inhibitory behaviour in all samples. Germination was less effective than roasting in reducing CIU; changes ranged from a slight increase for fababean, to 20-30% reduction for green pea and red lentil, 50-60% reduction for yellow pea, other lentils and chickpeas to approximately 90% for the beans and soybean. Germinated seed sere the most difficult to work with and showed the highest variability in the results. While there is variability the level of chymotrypsin inhibitors with respect to the pulse type, processing, particularly heat processing, reduces the level of this antinutritional factor to a point where it should not be a problem.

Introduction: Chymotrypsin inhibitors are routinely found in legumes, which includes pulses. While the concentration of chymotrypsin in pulses is often slightly lower that trypsin, the opposite is true for beans (Shi, 2015). Chymotrypsin inhibitors are similar in action to trypsin inhibitors, except they bind to chymotrypsin, rather than trypsin. As a result, the proteolytic and esterase activity of the chymotrypsin are blocked interfering with digestion and reducing the availability of essential amino acids (Savage, 1989). It is usually the combination of trypsin and chymotrypsin that interferes with protein digestibility. As both trypsin and chymotrypsin inhibitors are enzymes, there are sensitive to heat and it is expected that levels will be reducing when processing pulses for consumptions. The objective of this work was to evaluate the effect of boiling, roasting and germination on the levels of chymotrypsin in a range of pulse crops.

Materials and Methods: A variety of pulses including yellow and green peas, desi and kabuli chickpeas, red,French green, Spanish brown, medium green and large green lentils, dark red kidney, pinto, navy and black beans and fababeans were examined. For comparison soybean was also included. The processing treatments were boiling for 1h, roasting at 100 °C for 1 h at 12% moisture and germination by placing seed in an automatic sprouter for 3 days). Chymotrypsin inhibitory activity (CIA) was evaluated in the raw and processed seeds following the method of Makkar et al. (2007). One chymotrypsin unit was defined as an increase of 0.01 absorbance unit at 275 nm.

Results and Discussion: Chymotrypsin levels in raw pulses varied widely (1-24 CIU/mg), but all were less that what was found in soybean (30 CIU/mg). Beans had the highest CIU levels (18-24 CIU/mg) of all the pulses, but processing reduced the levels to below those found in other pulses. CIU levels were reduced by 83-90% with roasting, 90-93% with germination and completely eliminated with boiling (Table 1).

CIUs in raw chickpeas (~12 CIU /mg) were 4 times higher than in raw peas (~3 CIU/mg) and three times as high as raw lentil (~4 CIU/m); roasting greatly reduced CIU (93%) while the reduction due to germination was less (63-69%) (Table 1). Desi and Kabuli chickpeas responses were similar. As with the beans, boiling effectively eliminated all CIU activity. Raw lentil CIUs were higher than peas but less than chickpea and bean. Germination (24-60% reduction) and to a greater extent roasting (70-78% reduction) reduced these levels and boiling completely eliminated them. Red lentils appeared to retain the highest CIU levels after germination while both red lentils and medium green lentils retained slightly higher percentages of CIU when roasted (Table 1).

The CIUs were relatively low in peas. Both roasting and germination reduced CIU levels (Table 1); how greater reductions were observed for yellow peas. Again boiling eliminated all CIU activity. Raw fababean had the lowest CIU levels (~1CIU/mg), but less than half of this was destroyed with roasting and an increase was observed in the germinated sample. Boiling, however removed all CIU activity. Soybean, with the highest CIU, were most susceptible to processing with reductions of more than 90% for roasting and germination and complete removal with boiling.

Table 1. Percent reduction in chymotrypsin activity due to processing

	Roasted	Boiled	Germinated
Yellow Pea	72.76	100.00	52.63
Green Pea	46.91	100.00	29.15
Red Lentil	70.94	100.00	23.95
French Green Lentil	75.74	100.00	56.41
Spanish Brown Lentil	78.14	100.00	60.11
Large Green Lentil	76.30	100.00	56.80
Medium Green Lentil	70.91	100.00	51.37
Desi Chickpea	92.85	100.00	62.50
Kabuli chickpea	92.75	100.00	68.63
Faba	44.42	100.00	-12.17
Kidney	89.25	100.00	89.90
Pinto	83.30	100.00	93.01
Navy	87.96	100.00	90.13
Black	89.87	100.00	NA
Soybean	97.42	100.00	90.05

Conclusions: Chymotrypsin inhibitors are present in pulses, though levels are lower than in soybean. Boiling eliminated CIU in all crops. Roasting tended to be more effective than germination in reducing CIU levels (varied with crop). CIU in processed pulses are reduced to a point where they should not be a health issue.

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NP 23

EFFECTS OF PROCESSING ON PHENOLIC ACIDS IN PULSES

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Abstract: Concentrations of twelve phenolic acids were quantified from fifteen pulses to understand the effects of four treatments: raw, cooked, germinated and roasted. Free phenolic acids were extracted with acidified methanol (90:10; methanol: acetic acid). Bound phenolic acids were extracted using 2N NaOH, 10mM EDTA and 1% ascorbic acid (w/v). Extracted materials were quantified using HPLC. High levels of free gentisic acid were seen in beans, while the dominant free phenolic acids in lentils were ferulic and sinapic acid. Bound phenolic acid levels were generally higher. Ferulic acid was the main bound phenolic acid in beans; levels in other pulses were lower with gentisic and gallic acids being high in peas and lentils. For lentils, there was a shift from ferulic and sinapic acids in the free extract to gentisic and gallic acids in the bound material. Processing had little impact on free gentisic acid in pea and chickpeas, but germination resulted in higher levels in lentil and soybean. There was generally a loss of free gentisic acid in beans due to roasting and boiling. Processing beans also reduced the level of some bound phenolic acids, primarily the main one, ferulic acid. Losses of most phenolic acids were seen for soybean although the effect of processing varied with the phenolic acid and process. The value of the phenolic acids in legumes is primarily due to those which are bound. While processing had little impact on free phenolic acids, both heat and not-heat processing can adversely affect the level of the bound compounds.

Introduction: Phenolic compounds are widely distributed in the plant materials including pulses. Included in this group are phenolic acids, flavanoids and tannins, all of which contribute to the beneficial and antinutritional impact of these components. Phenolic acids in pulses can be either hydroxycinnamic (e.g.courmaric, caffeic, sinapic and ferulic acids) or hyxdroxybenzoic (e.g. vanillic, gallic, syringic, protocarhechric and p-hydroyxbenoic acids).

Phenolic acids are considered beneficial as they interfere with free radicals often associated with disease. However phenolic acids can adversely affect food taste and colour and some have been shown to interfere with trypsin and α-amylase activity (Shahidi et al., 2001).
The aim of this work is to examine the variability of free and bound phenolics acids in a range of pulses and examine how they are affected by processing.

Materials and Methods: Peas (yellow and green), chickpeas (Desi and Kabuli), lentils (Red, French Green, Spanish Brown, Large Green and Medium Green), Beans (Kidney, Pinto, Navy and Black) and Fababeans were evaluated in this study. Soybean was included as a control. Phenolic acids were evaluated in the raw beans as well as in seeds that had been boiled for 1h (high moisture) or roasted at 100 °C for 1 h at 12% moisture. A germination treatment (3 days in an automatic sprouter) was also included. Free phenolics were extracted using acidified methanol (90:10; methanol: acetic acid), while bound phenolics were extracted using 2N NaOH, 10mM EDTA and 1% ascorbic acid (w/v). The separation of the extracted phenolics acids was done using a Waters ACQUITY® Arc™ HPLC with a 2998 PDA Detector at 35°C and a flow rate of 1mL/min with a C18 Gemini® 5 µm column. Separation was attained using a gradient system of A: 1% formic acid in water and B: 1% formic acid in 100% methanol. The gradient went from 10% B to 30% B to 10% B in 30 min (Ross et al., 2009) Peaks were evaluated by comparison of retention times at 280 nm (gentisic acid 320 nm) to know standards (Table 1). Concentrations were determined based on external calibration curves.

Table 1. Average values for free and bound phenolic acids in raw pulses (µg/mg)

	Peas		Lentils		Chickpea		Fababean		Bean		Soybean	
	Free	bound	free	bound	free	bound	free	bound	free	bound	free	bound
Gallic	0.0	49.2	2.2	18.5	0.0	11.2	0.0	23.4	0.0	70.3	2.6	35.9
Protocatechuic	0.0	16.7	10.7	5.9	1.6	0.0	0.0	18.9	0.0	37.2	3.6	9.6
p-Hydroxybenzoic	0.0	0.0	4.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	35.0	0.0
Vanillic	0.0	0.0	0.8	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	176
Caffeic	0.0	16.9	3.6	0.0	0.8	0.0	0.0	0.0	0.0	25.2	0.0	33.7
Syringic	0.0	38.7	0.6	3.6	0.2	0.0	0.0	0.0	0.0	12.6	7.4	695
p-Coumaric	0.0	0.0	1.5	1.4	0.0	0.0	0.0	0.0	0.0	16.4	0.0	278
Ferulic	0.0	29.9	10.3	5.9	0.0	0.0	0.0	0.0	0.0	208	32.8	389
m-Coumaric	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sinapic	0.0	0.0	21.5	0.0	0.0	0.0	0.0	0.0	0.0	37.8	10.8	193
o-Coumaric	0.0	0.0	4.2	0.0	25.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gentisic	8.6	55.3	7.3	32.7	7.3	20.7	8.4	41.7	26.1	25.5	12.2	49.9

Results and discussion: The levels of bound phenolic acids (PA) were usually higher than free phenolics (Table 1). Gentisic acid (320nm) was present in all samples; other PA (280nm), were difficult to identify and if unidentified were not considered present. Ferulic acid was used as the internal marker, but when ferulic acid was not present, identification was a challenge. Bound gentisic acid was high in peas, chickpeas, lentils and fababean, while ferulic and syringic acids were high in beans and soybean, respectively (Table 1).

The distribution of PA was not the same for free and bound. In lentils, for example sinapic acid was dominant whereas gentisic acid was most prevalent for the bound phenolic acids. Heat processing had minimal effects on free gentisic acid in lentils, with only a slight increase due to germination. Boiling and roasting reduced free gentisic acid in pinto, navy and black bean and all processing methods reduced bound ferulic acid levels in beans (Fig. 5). The effect of processing soybeans varied for the different bound PAs. Roasting and boiling reduced coumaric, ferulic, vanillic and syringic acids while the effect of germination was minor. Gentisic acid increased with boiling and roasting but decreased with germination. Bound sinapic acid also increased as a result of roasting. Overall bound phenolic acids were sensitive to processing, although the effect varied with pulse type and the process.

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NP24

EFFECT OF DIFFERENT COOKING METHODS ON IN VITRO STARCH DIGESTION AND ESTIMATED GLYCEMIC INDEX OF COMMONLY CONSUMED PULSES

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Background and Objective

There is increasing interest in the use of pulses for producing healthier foods with low glycemic index. Previous studies with lentils have shown that *in vivo* glycemic response and GI are influenced by processing, and directly related to rapidly digestible starch (RDS) and inversely related to the slowly digestible starch (SDS) content. Given the increasing use of pulse products for the manufacture of "healthier" foods, it is important to determine whether the health benefits of pulses are retained during processing. The objective of the present study was to investigate the effect of four cooking methods on the *in vitro* digestion profiles of five commonly consumed pulses (red kidney and navy beans, chick and yellow peas and lentil) and to determine RDS, SDS, resistant starch (RScal) fractions, estimate glycemic index (eGI), and evaluate inter-relationships in commonly consumed pulses.

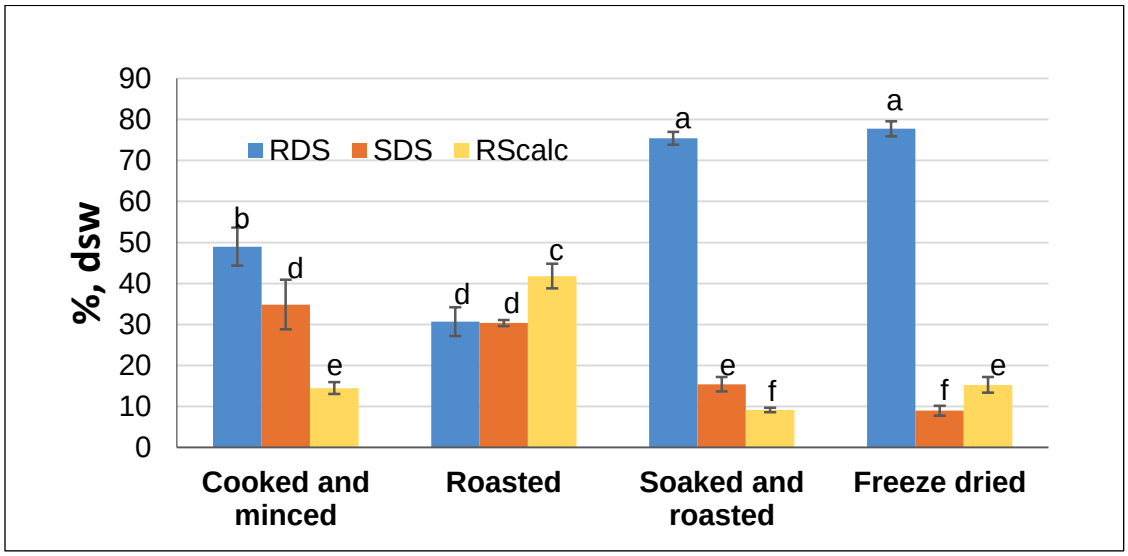
Methods

The four processing procedures examined include: freshly cooked then minced, which was either analysed immediately or freeze-dried; roasted in a convection oven (121°C x 25-50 min); and pre-soaked then roasted. Dry samples were milled and sifted to a standard particle size (250µm). Total starch content and resistant starch were determined using methods AACC 76-13 with 2 mL 90% DMSO pre-treatment and AOAC 2002.02, respectively. *In vitro* digestion was achieved by a simulated upper GI tract protocol (Englyst[1]) with modifications in a semi-automated instrument; glucose was determined directly @ 0, 20, 60 & 120 min using a glucose analyser (NutriScan GI20). Total Starch (%, dry weight basis) fractions were calculated as: RDS = % starch digested at 20 min, SDS = [% starch digested at 120 min] – [RDS], and RScal = 100 – [RDS+SDS]. Estimated GI was calculated according to Granfeldt et al. (1992)[2] using white bread as reference. Hydrolysis data from *in vitro* digestion was modelled and eGI calculated using white bread as reference. Microscopy (transmitted and polarized lights) analysis was also performed. All measurements were performed in triplicate and data was analysed using one-way ANOVA. Pearson correlation was used to assess the relationship between starch fractions and eGI.

Results

Roasted pulses had the lowest starch hydrolysis rates and associated eGI, followed by freshly cooked then minced samples. Freeze dried pulses and pre-soaked then roasted samples had the highest, and similar, starch hydrolysis rates and eGI. Among the roasted and the freshly cooked then minced samples, navy bean had the lowest eGI. Roasted pulses also had lower amounts of RDS than those prepared by the other 3 tested processing methods. Both roasted and fresh cooked then minced pulses had higher amounts of SDS. Pre-soaking prior to roasting increased the amounts of RDS and decreased SDS for tested bean and pea samples but not for lentil.

STARCH FRACTIONS IN PROCESSED RED KIDNEY BEAN:



Values are means±SD, n=3. Within each treatment means with different letters are significantly different ($p<0.05$)

Overall, RScal was higher for roasted pulses followed by freshly cooked and minced samples. *In vitro* eGI was related to RDS and derived RS content. Pearson Correlation between eGI and SDS or directly measured resistant starch was not significant.

Conclusion

Processing affects the in vitro digestion profile. Roasted pulse had the lowest eGI, followed by freshly cooked then minced, then by pre-soaked, then roasted, and lastly by freeze dried pulses which had the highest eGI. Collectively, these results suggest that processing may affect the biological efficacy of pulses. Differences in starch fractions and eGI resulting from cooking procedures may be attributed to changes in cell wall integrity and starch gelatinization. These results provide information that can help to guide the choice of production techniques for making food products that will retain the beneficial health characteristics that pulses are recognized for.

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NP25

A CORRELATION ANALYSIS BETWEEN DIFFERENT METHODS USED TO DEFINE PROTEIN QUALITY IN SOY AND CEREALS

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Background

Protein quality is the product of the protein content and its digestibility/availability for metabolic use[1]. Cereals tend to be limiting in one or more amino acids, thus impacting their overall protein quality. However, protein quality can be increased through blending cereal with complementary protein sources, including soy flours, which compensates for the limiting amino acids in the individual protein sources. Additionally, processing methods such as cooking are capable of modifying amino acid availability/digestibility and thereby protein quality[2].

Currently three methods of determining protein quality have been positioned, including the Protein Efficiency Ratio[1] (PER; Health Canada), the Protein Digestibility Corrected Amino Acid Score (PDCAAS; US FDA & WHO[1]) and the Digestible Indispensable Amino Acid Score (DIAAS; FAO proposal[3]). For this reason the objective of this study was to compare the estimates of protein quality derived via PER, PDCAAS and DIAAS for Canadian crops

Methods

In this study, estimates of protein quality were evaluated in an *in vivo* rat model using soy and cereal varietals. Sprague-Dawley rats (n=130, ~70g) were randomized to one of 13 diets corresponding to either cooked or raw Etna soy, Amadeus soy, Carberry wheat, Snowbird wheat, Turcotte oat and Navaro oat, with casein used as a control. The plant products were assessed for PER using a 28 day rodent bioassay adjusted using a reference value of 2.5 for casein; for PDCAAS using true fecal protein digestibility and the ratio of the limiting indispensable amino acid in the diet, using AOAC approved protocols. Ileal digestibility coefficients were derived from literature values and applied to the determined amino acid values of the plant products. Data were analyzed via correlational analysis.

Results

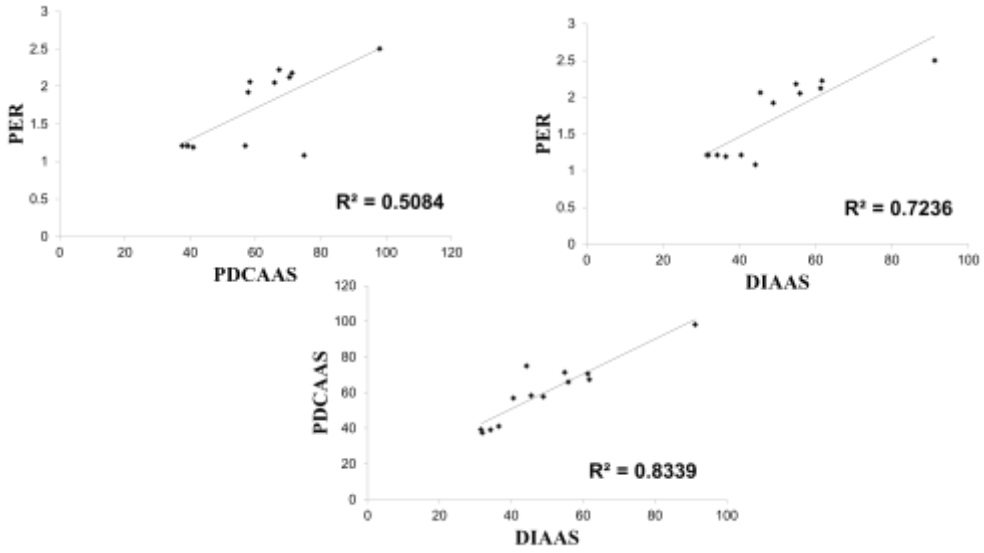
After feeding Sprague-Dawley rats with different diets it was determined that cooking led to higher PER values for soy flours, but did not impact the PER values of wheat or oat. Cooking also reduced the amino acid score (AAS) of the cereals. The cooked Snowbird wheat had the lowest PDCAAS (37.55%) and raw Etna soy the highest (74.96%). For DIAAS values raw Navaro oat had the highest (61.75%) and cooked Carberry wheat the lowest (31.57%). The findings of this study revealed a strong correlation between PER and DIAAS (R^2= 0.7913, p< 0.0002) and a significant correlation between PER and PDCAAS (R^2= 0.5075 p< 0.0062), as well as between PDCAAS and DIAAS (R^2= 0.8262, p<0.0001). PDCAAS may be higher than DIAAS due to the modifying effects of colonic microbiota. Furthermore, using a cut-off of 75 for DIAAS values would remove all plant proteins studied as sources of proteins. Additional inquiry is needed to clarify the potential risks associated with moving from one method of protein quality evaluation to another. Overall, further analyses are required for the determination of DIAAS, however these analyses are currently in process.

Table 1. Proximate analysis and protein quality determination via PER, PDCAAS and DIAAS.

				PDCAAS				DIAAS			
	Protein %	Dry matter %	PER	Limiting AA	AAS	Fecal N digest	Value	Limiting AA	AAS	Apparent Ileal N digest	Value
Casein	90.80	1.62	2.50	THR	1.02	95.99	97.81	M+C	0.91	86.35	91.25
Etna Soy Raw	36.39	1.63	1.08	M+C	0.88	83.51	73.25	M+C	0.44	56.37	44.29
Amadeus Soy Raw	44.58	1.64	1.21	M+C	0.70	82.20	57.38	M+C	0.41	64.66	40.57
Carberry Wheat Raw	14.12	1.90	1.19	LYS	0.46	89.34	41.13	LYS	0.37	84.01	36.51
Snowbird Wheat Raw	15.75	1.89	1.21	LYS	0.44	89.04	38.79	LYS	0.34	83.03	34.26
Turcotte Oats Raw	11.77	1.89	2.05	LYS	0.75	88.47	66.72	LYS	0.56	77.30	55.91
Navaro Oats Raw	11.38	1.77	2.22	LYS	0.76	87.70	66.43	LYS	0.62	84.70	61.75
Etna Soy Cooked	37.34	1.67	2.18	M+C	0.79	88.79	70.57	M+C	0.55	76.55	54.88
Amadeus Soy Cooked	45.86	1.61	2.12	M+C	0.78	89.69	70.32	M+C	0.61	86.12	61.34
Carberry Wheat Cooked	16.25	1.83	1.21	LYS	0.44	89.05	39.11	LYS	0.32	76.18	31.57
Snowbird Wheat Cooked	17.87	1.79	1.21	LYS	0.42	89.47	37.47	LYS	0.32	80.31	31.97
Turcotte Oats Cooked	13.89	1.69	1.92	LYS	0.66	86.89	57.38	LYS	0.49	77.14	48.94
Navaro Oats Cooked	13.90	1.58	2.06	LYS	0.68	85.13	57.65	LYS	0.46	70.26	45.53

Protein Efficiency Ratio (PER), Protein Digestibility Corrected Amino Acid Score (PDCAAS), Digestible Indispensable Amino Acid Score (DIAAS), Amino Acid Score (AAS)

Figure 1. Correlations between PER, PDCAAS and DIAAS.



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NP26

EFFECT OF CROP TYPE AND CROPPING LOCATION ON PLANT PROTEIN QUALITY USING AN IN VITRO MEASUREMENT OF DIGESTIBILITY

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Background

Environmental conditions and soil fertility can vary widely across cropping locations and this can impact the nutritional quality of crops grown in different fields. Differences in nutrient availability, absorption and deposition may also alter the nutritional quality of the protein derived crops grown in different areas. When considering protein quality the most important factors are the amino acid composition and the availability of those amino acids for metabolic work. Although the current recommendation is the use of *in vivo* experimentation for the determination of protein digestibility, this study utilized an *in vitro* digestibility assay, *in vitro* protein digestibility corrected amino acid score (IVPDCAAS), which has previously been demonstrated to have good correlation with *in vivo* data. This study investigated whether the protein quality of cereal and pulse proteins would differ if grown in two different locations. Although varietal differences in protein content have also been noted, this study purposefully used the same varietals across two different locations to minimize the effect of genetic variation.

Methods

For all samples, percent crude protein (CP; NX6.25) was determined through the use of a LECO CNS-2000 Nitrogen Analyzer. The amino acid contents of the samples were determined by acid hydrolysis using the AOAC Official Methods 982.30 (AOAC International). Methionine and cysteine were determined by the performic acid oxidized hydrolysis procedure, and tryptophan was determined using alkaline hydrolysis. Amino acid ratios for the samples were derived by dividing for each essential amino acid its relative abundance, expressed in milligrams of amino acid per gram of test protein, by the relative abundance of the same amino acid in the 1991 FAO adopted protein reference pattern. Amino acid scores were determined by selecting the value of the amino acid with the lowest ratio. For the determination of protein digestibility, 62.5 mg of protein was exposed to an enzyme cocktail consisting of trypsin, chymotrypsin and protease for 10 min and the resultant pH drop was recorded. The *in vitro* protein digestibility was calculated as follows, where the $\Delta pH_{10\ min}$ is the change in pH in 10 min from the initial pH of about 8.0

$$IVDP\% = 65.66 + 18.10 \cdot \Delta pH_{10\ min}$$

The IVPDCAAS was calculated as a product of the amino acid score and IVPD%

Results and Discussion

While there was little overall change in digestibility for oat, 0.45%, and wheat, 0.18%, differences were noted between locations for barley, 3.35%, and durum, 2.08% (Table 1). There was little change in the digestibility of the pulse crops investigated across locations, ranging from a difference of 0.35 % in soybean 4% in green pea. With respect to amino acid content, all cereal crops investigated were first limiting in lysine compared to the FAO/WHO recommendations, with wheat and durum also being limiting in threonine. Location did change the limiting amino acid for certain pulse classes, Desi chickpea (SAA vs thr), soybean (thr vs lys) and faba bean (SAA vs trp). When comparing the IVPDCAAS, only small differences were found for barley, 1.62, oat, 0.26, and wheat, 1.63, however the IVPDCAAS of durum differed by 8.78 between locations. This is due to the lower relative lysine content of one durum sample, amino acid score of 0.49 vs 0.62, despite

having a higher overall protein content, 15.41% vs 9.39%. While it may seem counterintuitive that higher protein content would result in lower relative lysine content, this has previously been demonstrated for both lysine and sulfur amino acids in field peas. In the pulse crops, IVPDCAAS remained relatively unchanged for the Kabuli chickpea, red lentil and green pea, however there were differences in Desi chickpea (19.58), yellow pea (7.97), soybean (11.37) and faba bean (18.0). In these cases the differences were primarily due to changes in the amino acid scores as the protein digestibility remained relatively consistent. Overall these is an indication that cropping location can alter both the protein content and amino acid composition of plant protein sources which will directly impact the nutritional quality of the resulting product. Additionally, these data suggest that pulse crops may be more susceptible to differences in cropping location than cereal crops.

Table 1: Protein content, amino acid scores *in vitro* digestibility and IVPDCAAS values for protein sources

	%CP[a]	Limiting amino acid	AAS[b]	IVPD[c]	IVPDCAAS[d]
Barley McGuire	11.41	LYS	0.71	75.8	53.48
Barley Star City	9.7	LYS	0.76	72.45	55.1
AC Morgan Oat	9.68	LYS	0.84	74.26	62.59
AC Morgan Blaine Lake Oat	10.55	LYS	0.84	73.81	62.33
Desi Chickpea	11.03	M+C[e]	1.23	74.62	91.74
Desi chickpea Elbow	22.6	THR	0.98	73.35	72.16
Wheat Lafleche	14.05	LYS	0.52	80.05	41.45
Wheat Melfort	11.88	LYS	0.5	80.23	39.82
Durum Sasil	15.41	LYS	0.49	80.77	39.77
Durum Herschel	9.39	LYS	0.62	78.69	48.55
Kabuli Moosejaw	15.66	M+C[e]	1.04	74.98	78.35
Kabuli Limerick	12.39	M+C[e]	1.02	74.35	76.09
Red Lentil Cutknife	25.21	M+C[e]	0.57	76.61	43.89
Red Lentil Saskatoon	24.03	M+C[e]	0.55	78.69	43.36
Yellow Pea S. Haunavan	21.72	M+C[e]	0.7	80.14	56.08
Yellow Pea Regina	21.79	M+C[e]	0.59	82.13	48.11
Green Pea North Battleford	22.26	M+C[e]	0.67	83.13	55.87
Green Pea Cudworth	21.88	M+C[e]	0.68	79.33	53.95
Soybean St. Dennis	36.58	THR	1.03	74.53	76.52
Soybean Carman	37.22	LYS	1.17	74.98	87.89
Faba Bean Saskatoon	28.39	M+Ca	0.61	75.89	46.19
Faba Beans St. Dennis	29.63	TRP	0.82	77.88	64.19

[a]%CP= % Crude Protein. [b]AAS= Amino Acid Score. [c]IVPD = In vitro protein digestibility.
[d]IVPDCAAS = In vitro protein digestibility corrected amino acid score. [e]M+C= methionine+cysteine.

NP27

EFFECT OF COOKING ON ANTI-NUTRITIONAL FACTORS EGG NOODLES SUPPLEMENTED WITH PULSE FLOUR

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Abstract:
Egg noodles supplemented with pulse flours were evaluated for trypsin inhibitors (TI), phytic acid (PA), total phenolic content (TPC) and antioxidant activity (AOX) before and after cooking. Blends that were 25% pulse flour and 75% were prepared with a variety of pea, lentil, chickpea, fababean and bean flours; an all wheat noodle was used as the control. Spectrophotometric methods were used for TI, PA and TPC analyses; AOX was based a DPPH (2,2-diphenyl-1-picrylhydrazyl) analysis which is based on a single electron transfer mechanism. Trypsin Inhibitors were most affected by cooking as dramatically lower values were seen for both the all wheat and pulse supplemented noodles. In comparison, PA levels remained the same or increased slightly upon cooking. The detected levels of TPC increased upon cooking for most of the doughs containing pulse flours whereas a decrease in TPC was obtained for the all wheat noodles. In contrast to the TPC values, DDPH values tended to be lower after cooking except for the all wheat noodle and the one supplemented with yellow pea flour where increased values were observed. This suggested the antioxidant activity was not entirely due to the phenolic compounds present. Egg noodles made with pulse flour showed both increased anti-oxidant and anti-nutritional activity compared to the control. The loss of TI, retention of TPC and relatively small reductions in AOX upon cooking are properties that are of value in noodles. The PA levels may be a concern and alternate methods to reduce PA may be necessary.

Introduction:
Use of pulse flours as ingredients in food products is one way to increase pulse consumption. However, pulse flours contain components that can be both beneficial (e.g. phenolic compounds) and antinutritional (e.g. trypsin inhibitors (TI) and phytic acid (PA)). Boiling has been shown to reduce TI and to a lesser extent PA when treating intact seeds (Shi, 2015). Reductions in specific phenolic acids have also been noted.

The objective of this work was to examine the effect of cooking (boiling) on TI, PA, total phenolic content (TPC) and antioxidant activity (AOX) when pulse flours are used as a food ingredient. Specifically, the changes during the boiling of egg noodles were examined.

Materials and Methods:
Pulses used in this study included yellow and green peas, desi and kabuli chickpeas, red, French green, Spanish brown, medium green and large green lentils, dark red kidney, pinto, navy and black beans and fababeans. Egg noodles were prepared by mixing 4 large eggs, 3½ cups flour (25% pulse flour, 75% all purpose flour) 1 tbsp water and 1 tsp salt until the mixture appeared crumbly. It was then hand-kneaded for 30 to 60s to produce a smooth, pliable dough. Noodles were prepared with a Shule manual pasta maker and cooked by boiling for ~5 min. An all-wheat control was used for comparison. Raw and cooked noodles were analyzed for trypsin inhibitors (Kakade et al., 1974), phytic acid (Latta and Eskin, 1980), total phenolic content (Gao et al., 2002) and antioxidant activity based on a DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Chen and Ho, 1995).

Results and Discussion:

Trypsin inhibitor activity (TIA) was reduced by cooking for all samples; TIA was below detection limits for cooked red lentil, medium green lentil, Spanish brown lentil and Desi chickpea. Phytic acid (PA) in pulse based noodles remained the same or increased slightly as a result of cooking; slight increases were observed for medium green lentil, fababean, pinto bean and black bean. For most pulse based noodles, total phenolic content (TPC) increased slightly due to cooking; navy bean was an exception. A decrease in TPC was noted for the all-wheat noodle. Antioxidant activity (AOX), as measured by DPPH, remained essentially the same (yellow and green peas and navy beans) or decreased upon cooking for pulse-based noodle samples; an increase in AOX was noted for the wheat sample (Table 1).

Table 1. Levels of trypsin inhibitors (TIU), phytic acid (PA), antioxidant activity (AOX) and total phenolic content (TPC) in raw and cooked noodles contain pulse flours.

Pulse	TIU/mg		PA (mg/g)		TPC (µmol DE/100g)		AOX(µmol TE/100g)	
	raw	cooked	raw	cooked	raw	cooked	raw	cooked
Yellow pea	5.45	0.80	0.85	0.82	119	192	66	117
Green pea	5.13	0.54	0.79	0.69	112	196	72	77
Red lentil	6.02	0.00	1.19	1.06	344	455	850	609
French green lentil	5.78	0.50	1.17	1.19	346	507	749	640
Large green lentil	6.12	0.61	0.85	1.02	306	395	716	620
Medium green lentil	6.09	0.00	0.95	1.35	291	415	733	563
Spanish brown lentil	5.88	0.00	1.1	1.23	283	402	765	492
Desi chickpea	7.23	0.00	1.01	0.98	196	239	191	112
Kabuli chickpea	7.85	1.08	0.96	0.89	116	204	81	55.5
Faba bean	5.67	0.72	1.35	1.63	409	582	861	596
Pinto bean	8.84	1.03	1.33	1.72	356	451	741	433
Navy bean	9.81	2.09	1.32	1.15	195	180	87	113
Red kidney bean	9.07	0.86	1.21	1.2	293	358	609	324
Black bean	9.39	0.79	1.19	1.49	203	271	199	217
Wheat	4.07	0.80	0.34	0.34	129	75	35	76

Conclusions:

In a food matrix, egg noodles, the effect of cooking was similar to that reported for boiling of the flour (Shi, 2015); trypsin activity decreases while changes in phytic acid are limited. The antioxidant activity associated with pulses does not appear to be due to phenolic content alone, as antioxidant activity decreased with cooking despite an increase in total phenolics.

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NP28

EVALUATION OF THE ADDITION OF PEA FIBRE FRACTIONS ON DOUGH PROOFING POTENTIAL

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Introduction

There is a growing emphasis on improving health via diet. Therefore, bread manufacturers are interested in strategies that will enrich products with ingredients such as fibre that enhance the nutrient profile of baked products. However, fibre usually negatively affects bread quality. Possible mechanisms include puncturing of gas cells, interfering with gluten development, and effects on dough viscosity.

The aim of this study was to examine the effect of yellow pea hull fibre on bread quality arising from fibre interactions with bubbles in the dough in order to understand the physical effects of pea fibre on gas cell structure in the proofing loaf.

Materials & Methods

Four particle sizes of pea fibre were used: 180, 125, 106, 90 µm, added to create five addition levels in the bread: 0, 2, 4, 6, 8 g/serving (determined according to Health Canada labelling regulations regarding dietary fibre; e.g., 2 g/serving = “source of dietary fibre”). The 125 µm fraction was directly sourced from Best Cooking Pulses, with other particle sizes milled from pea hull bran.

A sponge and dough method was used for baking using CWRS wheat flour (13.3% protein). Water absorption was not altered initially, but a second set of experiments was conducted where bread was baked with optimal water levels (determined using a farinograph).

An ultrasonic broadband transducer was used in reflection mode to measure ultrasonic signals in dough as a function of time during proofing. Specific loaf volume and crumb quality (C-cell analysis) were evaluated in the resulting loaves.

Results & Discussion

The effect of increasing pea fibre on specific loaf volume depression was evident for all particle sizes when water content was maintained at constant levels. Quality depression was particularly noticeable for small particle sizes and higher fibre loadings (illustrated for 180 µm fraction, Fig. 1).

Fig. 1. Loaves baked with increasing amounts of 180 µm pea fibre, left to right: 0, 2, 4, 6, 8 g/serving.

The depression of specific loaf volume with increasing fibre content and decreasing particle size was reflected in a reduction in crumb gas cell diameter (Fig. 2).

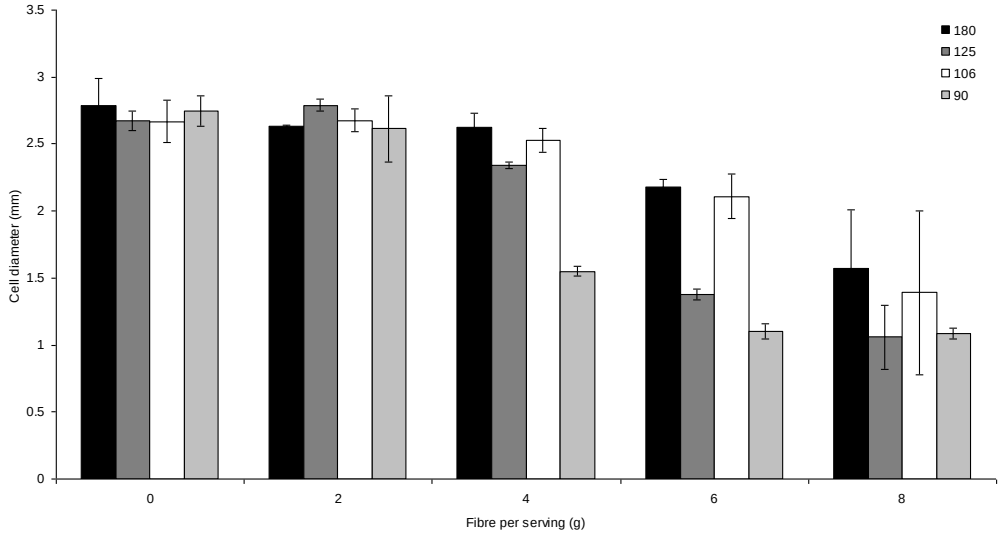
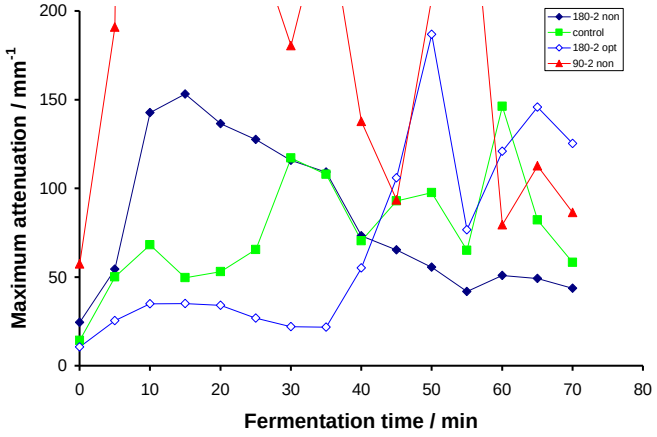


Fig. 2. Effect of fibre loading and particle size on gas cell diameter for doughs prepared at fixed water levels.

C-cell crumb analysis also demonstrated (not shown here) that for a given fibre loading, gas cell size homogeneity increased as particle size decreased. This suggested that volume depression arose from high dough viscosity limiting gas cell expansion rather than from fibre particles promoting gas cell coalescence. To examine if viscosity effects were manifest in the proofing dough, ultrasonic analysis was conducted in the proofing chamber. Analyses were conducted on both optimized and non-optimized water absorptions (Fig. 3).



Ultrasonic profiling during fermentation showed a spike at early time in the attenuation of doughs where water content was not optimized. Water content optimization led to a delay in the time where peak attenuation was at its maximum. This analysis indicates ultrasound's potential to identify quality defects associated with non-optimized fibre formulations during dough proofing.

Fig. 3. Change in maximum attenuation coefficient value with fermentation time for doughs (optimized or non-optimal water absorption) with different fibres (at 2g per serving loading) in comparison to control.

Conclusions

In both optimized and non-optimized loaves, the effect of pea fibre on bread quality is due to enhanced dough viscosity rather than to greater gas cell coalescence arising from fibre particles interacting with bubbles in the dough in the proofing stage.

Acknowledgements: NSERC Canada, Best Cooking Pulses Inc. and Agri-Food Research and Development Initiative.

NP29

PULSE PUREES: AN ARSENAL OF CONSISTENCIES (TEXTURES) FOR PRODUCT DEVELOPMENT OPPORTUNITIES IN THE FOOD INDUSTRY

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Introduction

The high protein and high fibre properties of pulses make them an attractive ingredient for product development strategies in the food industry. Nevertheless, there can be significant quality challenges when adding whole pulses or pulse fractions to food products. This is particularly true with respect to the hydration needs of the fibre component and its potential to withdraw moisture from other components leading to quality defects in the reformulated product. One potential means of addressing hydration challenges in product development is to use pureed pulse products so that the moisture migration issues are eliminated. If purees are available in a range of consistencies (textures) as aseptic offerings in a shelf-stable format, pulses and pulse fractions can be developed into an array of readily usable product development opportunities with a reduced environmental footprint. Our objective was to quantify the changes in flow properties of white navy bean and chickpea purees produced to have a range of consistencies.

Materials & Methods

A pilot-scale steam infusion aseptic thermal sterilization process on pulse flours (Best Cooking Pulses, Portage-la-Prairie, MB) was used to manufacture a suite of shelf-stable white navy bean (NC) and chickpea purees (CA,CB,CC,CD). Chickpea purees are shown in Fig.1.

A rheometer (AR2000, TA Instruments, New Castle, USA), was used to quantify the changes in puree consistency under shear flow and oscillatory modes.

Fig. 1. Chickpea purees with different consistencies (left to right: CA, CB, CC and CD).

Results & Discussion

Under shear flow testing, pulse purees exhibited non-Newtonian behavior (essentially shear-thinning). Massive differences in the consistency of the purees showed up as contrasting flow curves (Fig. 2, note log scale). The navy bean and three of the chickpea purees exhibited a yield stress, so that they possess a solid-like character, requiring a minimum stress to start flowing.

	White Navy Bean	Chickpea (CA)	Chickpea (CB)	Chickpea (CC)	Chickpea (CD)
Yield Stress / Pa	223	0	4	96	98

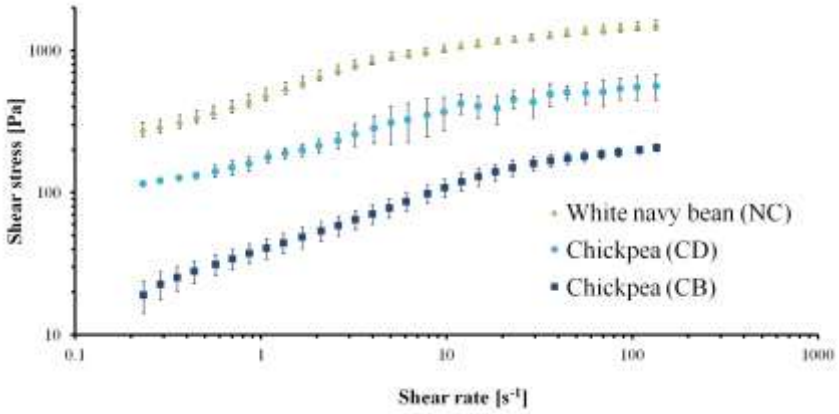

Fig. 2. Shear stress vs. shear rate of white navy bean and two selected chickpea purees.

Under oscillatory testing, the complex viscosity of white navy bean puree was greater than any of the chickpea purees (Fig. 3). The complex viscosity decreased with increasing angular frequency, except for CA (the chickpea puree with the runniest consistency). Thus, oscillatory testing confirmed shear flow evaluations that most pulse purees were shear-thinning materials.

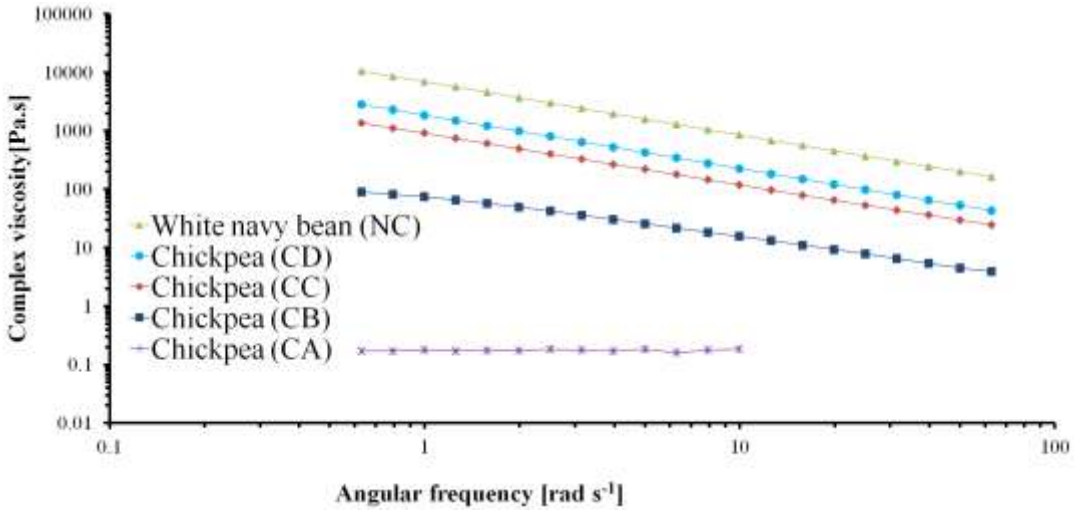

Fig. 3. Complex viscosity against angular frequency for white navy bean and four chickpea purees.

Conclusions

Pulse purees show non-Newtonian behavior with their viscosity decreasing with increasing shear rate (shear-thinning).

White navy bean puree was more solid-like and had harder to flow properties than any chickpea puree.

The wide range in consistencies in an aseptic shelf-stable format opens up a range of product development options using pulse puree ingredients from meat extenders to hummus to desserts.

NP30

SCALING UP PULSE INNOVATION IN SOUTHERN ETHIOPIA: VIRTUES AND CHALLENGES

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Within the last decade scaling-up has emerged as a crucial strategy to expand the scale and improve the impact and effectiveness of development intervention. This study demonstrates some of the reasons for the success of a scaling up of pulse innovation for food and nutrition security in Southern Ethiopia. First, the study develops a critical framework to analyse some of the key elements and processes that allow scaling up to happen. It then uses the framework and the data from an empirical research undertaken on the pulse innovation project in Southern Ethiopia to show some of the reasons for the success in scaling up pulse innovation in Southern Ethiopia. In explicating the process of scaling up, the study outlines institutional linkages and lessons learned from rigorous evaluations. The study also documents some of the challenges that limit the magnitude of success of the scaling up process and praxis.

NP31

EFFECT OF EXTRUSION AND STORAGE ON PHYSICOCHEMICAL PROPERTIES AND NUTRITIONAL COMPOSITION OF CHICKPEA-MAIZE AND CHICKPEA-SORGHUM SNACKS

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Chickpea has been used as a blend component in the production of extruded snacks in a number of studies. However, the effect extrusion and storage on physicochemical characteristics and nutritional composition of chickpea-maize and chickpea-sorghum snacks has not been investigated. In this study, the effect of extrusion temperature (150 and 170 °C), feed moisture content (15, 17 and 20%) and storage time (0, 15 and 30 days) on physicochemical and nutritional composition of 30:70 blend of chickpea-maize and chickpea-sorghum snacks was investigated using factorial design. It was found that extrusion temperature and moisture content have significant effect ($P<0.05$) on expansion ratio and hardness of the snacks. Chickpea-maize snacks at 170 °C and moisture content of 15 and 17% scored higher expansion ratio of 4.54 and 4.55 while chickpea-sorghum snacks at 150 °C and moisture content of 15 and 20% scored better expansion ratio of 3.15 and 3.13. Chickpea-maize snacks at 150 °C and moisture content of 15% scored lowest hardness value of 41.87 N while chickpea-sorghum snacks at 150 °C and 20% scored 76.65 N. Extrusion temperature and storage time significantly affected ($P<0.05$) peroxide value, tannin and protein content of chickpea-maize snacks. Storage time affected fat, carbohydrate and energy contents of the snacks.

NP32

ACCEPTABILITY AND FEASIBILITY OF A NUTRITION INTERVENTION TO PROMOTE CONSUMPTION OF PULSE BASED FOOD PRODUCTS IN CHILDCARE CENTERS IN SASKATCHEWAN

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Objective: The study's objective was to evaluate the acceptability and feasibility of a pilot pulse based nutrition education intervention titled a 'Pulse Discovery Tool Kit.' The kit was designed to promote healthy eating habits and pulse consumption in childcare centers.
Method: The study was conducted in two childcare centers in Saskatoon, Saskatchewan. A pre-post 12-week intervention design was employed to pilot test the Pulse Discovery Tool Kit among children (2 to 5 year olds).The intervention included weekly lesson plans, taste testing, and parent's newsletters. Data was captured through questionnaires, sensory evaluations, pulse knowledge test, lesson plan evaluations, cook questionnaires and semi-qualitative interviews. A pulse knowledge score was constructed based on their response to pulse knowledge questions.
Results: Preliminary results indicated that there was a significant improvement in the mean 'pulse knowledge score' of the children at post intervention (1.68) compared with their score at baseline study (a score of 1) ($p=0.033$). Sensory analysis indicated the percentage of children that liked green split pea spread was 44% which increased to 56% during repeated taste testing.
Conclusion: The Pulse Discovery Tool Kit showed good initial acceptability and feasibility among the study population.

NP33

EFFECTS OF SEED TEMPERING AND MICRONIZATION TEMPERATURE ON THE FUNCTIONAL PROPERTIES AND DIGESTIBILITY OF DESI CHICKPEA FLOUR

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Chickpeas are an important pulse crop around the world, as they represent a good source of protein and other macro-/micro-nutrients. For this research, the effects of processing (i.e., infrared heating or micronization, with and without tempering) on the protein quality within desi chickpea flour was examined. Micronization is a short time, high intensity, infrared heating process used to reduce the cooking times of pulses. Penetration of infrared rays into the seeds causes the vibration of water molecules and a consequent rapid internal heating and rise in water vapor pressure inside the material, resulting in protein denaturation and starch gelatinization. The process can also reduce the levels of volatile compounds that are responsible for some of the undesirable sensory attributes of pulses. Tempering prior to infrared heating allows moisture to penetrate the seed creating a more porous structure. The higher amount of moisture can lead to the generation of more heat when processed and also some losses in soluble antinutritional compounds.

Objective:

The overall objective of this research was to investigate the impact of tempering moisture and seed surface temperature generated by infrared heating on the physicochemical and functional properties and the protein quality of chickpea and barley flours.

Methods:

Desi chickpeas (var.: CDC Consul (dehulled/split) grown in Elbow, SK in 2014) were kindly donated by Diefenbaker Seeds (Saskatoon, SK). Seeds were left either untempered or tempered to 20% moisture prior to infrared heating. Infrared heating was carried out at InfraReady Products (1998) Ltd. (Saskatoon, SK) using a laboratory scale micronizer. Approximately 2 kg of each tempered and untempered sample was processed in order to reach the surface temperatures of 115^{o}C and 135^{o}C. Each heating treatment was carried out under the same condition three times to achieve three processing replicates. All processed seeds were dried to moisture levels <10% using the laboratory scale micronizer. Seeds were then ground into coarse flour using a disc mill, and then into finer flour using a UDY Cyclone Sample Mill. Samples were assessed for their proximate composition, functional attributes and *in vitro* digestibility.

Results:

The proximate composition for untreated and treated desi chickpea flour was similar, having ~25% protein (d.b., dry basis), ~3% (d.b.) ash, ~5% (d.b.) crude fat and ~42% (d.b.) total starch. The presence of antinutritional compounds can have an adverse effect on protein digestibility For instance, trypsin inhibitor activity can reduce the ability for proteins to be cleaved by digestive enzymes, whereas phenolic compounds or condensed tannins are able to cross link proteins to inhibit their unfolding and digestion. The levels of total phenolics within desi chickpea flours were found to significantly decline from 17.1 g catechin equivalent/100 g of flour (d.b.) for un-tempered non-micronized seeds to 14.3 g catechin equivalent/100 g of flour (d.b.) for seeds tempered to 20% moisture and then heated to a surface temperature of 135^{o}C. The decline in total phenolic compounds suggests that they are heat liable. In contrast, condensed tannins levels within the chickpea flours were almost negligible, which is attributed to the use of dehulled split chickpeas as

the feedstock. In terms of the trypsin inhibitor activity, values declined from ~16.3 TIU/ mg of flour (d.b.) for un-tempered non-micronized seeds, to 14.9 and 9.3 TIU/ mg of flour (d.b.) for seeds brought to a surface temperature of 115^{o}C and 135^{o}C, respectively. The addition of 20% tempering plus heating to either a surface temperature of 115^{o}C or 135^{o}C resulted in a further decline in trypsin inhibitor activity to ~3.7 and ~2.7 TIU/ mg of flour (d.b.).

The water hydration/oil holding capacities, foaming abilities and emulsifying properties were investigated for all samples. Water hydration capacity increased from the un-tempered non-micronized flours (1.1 g/g) to ~1.3 g/g and ~1.6 g/g with the addition of heat to 115^{o}C and 135^{o}C, respectively. Water hydration capacity increased further to ~1.7 g/g and ~1.8 g/g with the combination of tempering to 20% moisture plus heat to 115^{o}C and 135^{o}C, respectively. In contrast, no significant differences were found in the oil holding capacity data. Emulsion activity values were found to increase significantly from 44.5% in the case of un-tempered non-micronized desi chickpea flour to 47.1% in the case of flour derived from seeds tempered to 20% moisture and heated to a surface temperature of 135^{o}C. The rise in the emulsion forming properties is believed to be associated with the unraveling of the protein structure to expose previously buried hydrophobic amino acids. However, once the emulsions were formed, emulsion stability was found to be ~48% regardless of the treatment. Un-tempered non-micronized desi chickpea flours had a foam capacity of ~181%, which increased to ~217% when heated to a surface temperature of 115^{o}C, before then declining to ~186% when heated to 135^{o}C. When seeds were tempered to 20% moisture and heated (115/135^{o}C), foam capacity declined further to ~130%. It was hypothesized that the initial rise in foam capacity when seeds were heated to 115^{o}C was attributed to the partial unfolding of the protein structure, where if too much denaturation occurred (with higher processing), the proteins would have issues migrating to the air-water interface. Un-tempered non-micronized desi chickpea flours had a foam stability of ~85%, which increased to ~93% with the addition of heat (115^{o}C). Foam stability subsequently declined when heated to 135^{o}C (~89%), or with tempering to 20% moisture with heating to 115^{o}C (~84%) or 135^{o}C (~79%).

Based on the amino acid scores, desi chickpeas were found to be limiting in threonine. Overall, the addition of heat and tempering + heat resulted in increased *in vitro* digestibility relative to the raw flour; however temperature or moisture levels did not have a significant effect. *In vitro* protein digestibility corrected amino acid scores were found to increase from 63-66% for untreated flour, heated flour, or tempered to 20% moisture + heating at 115^{o}C, to ~71%, when tempered to 20% moisture with heating to 135^{o}C.

Conclusions:

Overall, tempering in combination with infrared heating resulted in a higher quality protein, associated with improved digestibility, lower levels of antinutritional compounds, and slightly improved protein functionality.

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NP34

EFFECT OF EXTRUSION CONDITIONS ON CHICKPEA AND CEREAL FLOURS, AND THE DIGESTIBILITY OF THEIR BLENDS

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Extrusion is a continuous high temperature short time (HTST) process, during which the material is pushed by a piston or a screw under pressure and shear through a die with a given shape. This process combines pumping, mixing, kneading, heating and cutting all in one place, and has been widely used in the food industry for cereals, snacks, pet food, animal feed and more (1). Because of the HTST processing, the nutritional loss of vulnerable components such as protein, vitamins and enzymes in the extruded foods is held at a minimum, and some antinutritional factors are destroyed during the process. Barrel temperature and moisture content have a great effect on the physicochemical, nutritional, functional and sensory properties of the extrudates (2). Traditionally, pulse crops are consumed along with cereals for complementary nutrition, since pulse proteins are high in lysine and limited in thiol containing amino acids (methionine & cysteine), and cereals are richer in thiol containing amino acids but limited in lysine. Kabuli chickpea, sorghum and maize are three gluten-free crops that have been rarely studied in blended extrusion applications.

Objective:

The overall goal of this research was to investigate the impact of extrusion conditions on the physical properties of kabuli chickpea, sorghum and maize flours, as well as the in vitro protein digestibility of blended unprocessed flours.

Methods:

Kabuli chickpea, sorghum and maize flours were extruded using a twin screw extruder as a function of barrel temperature (120, 135 and 150^{o}C) and percent moisture (20, 22 and 24%). The resulting physical properties of the extrudates were examined, including hardness, bulk density, expansion ratio and the specific mechanical energy. Further, essential amino acid profiles, *in vitro* protein digestibility and *in vitro* protein digestibility corrected amino acid scores (IV-PDCAAS) were determined for chickpea-cereal blends at ratios of 50:50, 60:40, 70:30 and 80:20 using un-extruded flours.

Results:

Chickpea extrudates were shown to have the highest hardness and bulk density values at all pre-set extrusion conditions relative to the sorghum and maize extrudates. In the case of maize, the highest and lowest bulk density values were observed at 120^{o}C/24% moisture and 120^{o}C/20% moisture, respectively, whereas the highest and lowest hardness values were observed at 120^{o}C/24% moisture and 135^{o}C/20% moisture, respectively. In the case of sorghum, the highest and lowest bulk density values were observed at 120^{o}C/24% moisture and 150^{o}C/20% moisture respectively, whereas the highest and lowest hardness values were observed at 120^{o}C/22% moisture and 150^{o}C/20% moisture, respectively. Overall, increases in temperature significantly a) decreased the expansion ratio of maize extrudates; and b) decreased the specific mechanical energy, hardness and bulk density of sorghum and chickpea extrudates. Overall increases in moisture significantly a) increased the bulk density and hardness of all three extrudates; b) decreased the specific mechanical energy of maize and sorghum extrudates; c) and decreased the expansion ratio of both maize and sorghum extrudates.

Sorghum and maize flours (unextruded) were found to have lower values for each essential amino acid (EAA) compared to chickpea, and had corresponding limiting amino acid scores of 0.35 and 0.56, respectively associated with lysine. In contrast, the limiting amino score for chickpea was 0.94 which was for threonine. An interesting finding was that chickpea from the 2015 crop year in Saskatchewan appeared to be almost nutritionally complete in terms of its essential amino acid composition, and had an amino acid score for the thiol containing amino acids of 0.98. The higher amount of cysteine and methionine present is hypothesized to be as the result of soil fertility practices involving canola. During crop rotation years, it was presumed that chickpea was able to uptake certain compounds from the soil and synthesize it into amino acids. Because of the higher than expected levels of thiol containing amino acids in the chickpea, blending with cereals, which would normally have a positive effect on nutrition, actually had a negative effect by lowering the limiting amino acid scores in all cases from the chickpea alone, regardless of the blending ratio. For all flours and blended flours, *in vitro* digestibility values ranged between ~81 to 85%. IV-PDCAAS values for chickpea, sorghum and maize flours were 80%, 29% and 46%, respectively. Similar to the limiting amino acid scores, blending also had an effect of lowering the IV-PDCAAS values, due to the chickpea flour. In the case of chickpea-sorghum blends, IV-PDCAAS scores were 69%, 77%, 75% and 79% for ratios of of 50:50, 60:40, 70:30 and 80:20, respectively. In the case of chickpea-maize blends, IV-PDCAAS scores were found to be 77%, 78%, 75% and 80% for ratios of of 50:50, 60:40, 70:30 and 80:20, respectively.

Conclusions:

Extrusion conditions, such as barrel temperature and % moisture can be used as a means for tailoring the extrudates to specific physical properties. Chickpea flour appeared to be almost nutritionally complete in terms of its essential amino acid profile, where blending with cereal flours was shown to have a negative on IV-PDCAAS values. However, it is presumed that chickpea flours would contribute to a much greater extent to the bulk density and hardness of blended extrudates possibly offering an advantage for creating a superior product, despite being slightly less nutritious in terms of protein quality.

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