MANITOBA
Pulse Soybean
GROWERS

pulse beat

The Science Edition

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In this issue

On-Farm Network • Yield & Quality • Pest Control • Market Demand

Developing production tools and market demand for profitable and sustainable farms through local research.

The Manitoba Pulse & Soybean Growers (MPSG) research program has a lineup of projects that support our goal of reducing the economic consequences of crop pests. Researchers in pathology, entomology and weed science have been experimenting with practical options for fungicide application and crop rotation. The latest results from this work are featured in this edition of *Pulse Beat – The Science Edition*. Of course, there's more to farming than contending with pests. So, by extension, there's more to research than bugs and weeds. At winter meetings, we've been emphasizing the short history of annual legume production in Manitoba. One result is that we know little about the turnover of soil microbes and nutrients in rotations containing annual legumes. In this regard, Dr. Ivan Oresnik at the University of Manitoba reports on his observations on the fate of soybean inoculant in the soil. Also, a few years back, MPSG invested in research to improve the prospect of value-added processing of pulse and soybean crops. As this edition's reports show, these farm products are indeed suitable for a variety of applications in the food industry. A report on MPSG's foray into drought-tolerance research takes us into the world of plant physiology and the search for processes in the plant that can be channeled into buffering yields against drought. Back on the farm, reports are in on avoiding seed damage by air seeders and on safely storing soybeans.

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On-Farm Evaluation of Fungicide in Soybeans

Fungicide application at first bloom (R1) to full flower (R2) significantly increased soybean yields 14% of the time. Soybean yield was not frequently limited by Septoria brown spot or white mould in these trials.

THE ON-FARM NETWORK (OFN), MPSG's in-house research program, began investigating soybean yield response to fungicide application in 2014. Over the past six growing seasons, there have been a total of 59 soybean fungicide trials across the province using randomized and replicated strip trials in farmers' fields. These trials compared soybean yield with and without a single foliar fungicide application intended to control fungal diseases, including Septoria brown spot, frogeye leaf spot and white mould. Product choice (e.g., Acapela, Cotegra, Delaro, Priaxor) was at the discretion of the farmer, and all applications were made according to label rates at the recommended timings of R1 (beginning bloom) or R2 (full flower).

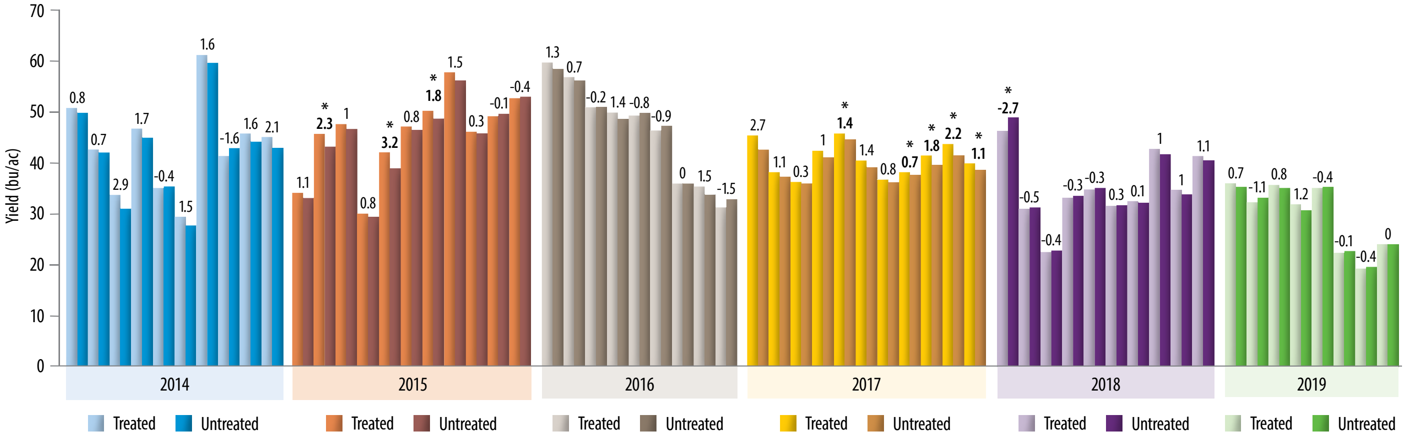
Among the 59 trials, there were only nine statistically significant yield responses to fungicide application, eight of which were positive. This means fungicide application significantly increased soybean seed yield 14% of the time. Additionally, all significant responses occurred in three of the six growing seasons – 2015, 2017 and 2018 (Figure 1). Septoria brown spot and white mould disease pressure were also reduced by fungicide in most responsive trials.

The variability of yield benefits from fungicide application across growing seasons is not a surprise. The extent and severity of disease pressure is typically inconsistent across years and dependent on growing season conditions. Fungicide application is also expected to protect yield only when fungal disease pressure is significant enough to be limiting.

Infrequent soybean yield response to foliar fungicide application (14%) is also not surprising. In Manitoba, soybean yield is not often limited by Septoria brown spot, frogeye leaf spot and white mould. This disease pressure was low overall within responsive trials, minimizing our expectation of a yield response from fungicide. Farmers' input decisions involve a number of factors, including balancing the cost of product and application with the risk of yield loss from pest pressure. Consideration should also be given to the expected frequency of a positive outcome. This means a given yield increase from fungicide must pay for itself in the current successful year, and also pay for prior years in which the product did not improve yield.

For more information on each of the OFN soybean fungicide trial sites and results, visit manitobapulse.ca/on-farm-network.

Figure 1. Yield difference (indicated by the value above the paired bars) between soybeans with foliar fungicide applied (treated) and soybeans without foliar fungicide (untreated) for individual On-Farm Network trials from 2014–2019.



Numbers above bars indicate yield differences between soybeans with a single foliar fungicide application and untreated soybeans. * Statistically significant yield difference at $p < 0.05$.

PRINCIPAL INVESTIGATOR Manitoba Pulse & Soybean Growers – On-Farm Network

MPSG INVESTMENT $255,733

DURATION Ongoing

On-Farm Evaluation of Fungicide in Dry Beans

There was no significant yield response to foliar fungicide application in dry beans across all 15 trials conducted from 2016 to 2019. When disease pressure is low, fungicide application is not expected to provide a significant yield benefit.

WHITE MOULD MANAGEMENT is an important consideration for dry bean farmers in Manitoba. White mould is generally managed with foliar fungicide applications around the R2 (early pin bean) stage. MPSG's On-Farm Network (OFN) began investigating dry bean response to a single foliar fungicide application in 2016. Since then, a total of 15 replicated and randomized trials have been conducted through the OFN (Figure 1). Each trial compared untreated dry beans to those with a single foliar fungicide application of a product of the farmer's choice (e.g., Acapela, Cotegra, Lance), applied according to label recommendations.

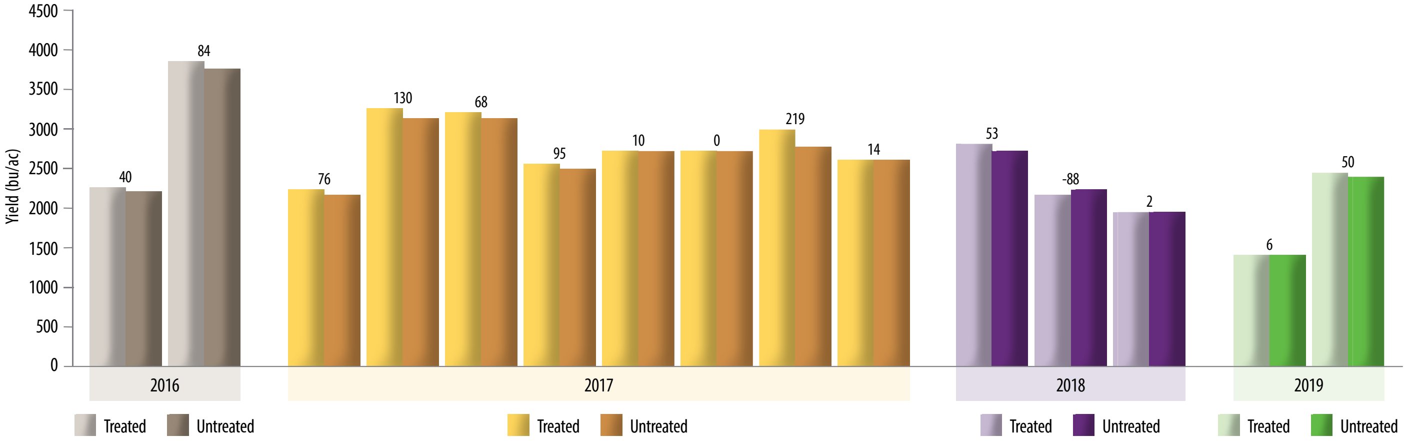
According to the 15 trials conducted to date, there have not been any significant yield differences between dry beans with a foliar fungicide application and those without a foliar fungicide application. Disease pressure ratings were taken mid-season at each location, shortly after fungicide application timing, to assess disease pressure differences between dry beans with and without fungicide. Disease pressure was low overall.

At low disease pressure, fungicide is not expected to have a significant effect on yield. In years with conditions that are conducive to white mould development, resulting in greater incidence and severity of the disease, the yield outcomes with and without fungicide application could significantly differ. For this reason, the OFN is continuing these trials to capture dry bean response to fungicide application under different weather conditions and across more growing seasons.

The lack of yield response at all 15 of these trials indicates the importance of evaluating the risk of white mould during each growing season. "Insurance" application of fungicide can be costly and evidently does not provide any yield benefits when disease pressure is not yield-limiting. Considerations for whether to apply fungicide or not, include weather conditions (rainfall, humidity, temperature) both leading up to flowering (at the V4 stage or 1–2 weeks prior to fungicide timing) and during flowering, rotation with other host crops of white mould, row spacing, plant density and varietal susceptibility.

A fungicide decision tool for managing white mould in dry beans can be found at manitobapulse.ca or in the MPSG Bean App.

Figure 1. Yield difference (indicated by the value above the paired bars) between dry beans with a single foliar fungicide application and untreated dry beans for each trial from 2016–2019.



Numbers above bars indicate yield differences between dry beans with a single foliar fungicide application and untreated dry beans.

PRINCIPAL INVESTIGATOR Manitoba Pulse & Soybean Growers – On-Farm Network

MPSG INVESTMENT $56 659

DURATION Ongoing

Evaluation of Fungicide Options to Control White Mould in Pinto Beans

All fungicides improved pinto bean yields in two out of three years when white mould pressure was high. The break-even yield to cover the cost of fungicide varies depending on the cost of the product and the yield response.

WHITE MOULD (*SCLEROTINIA*) is the number one disease concern for dry bean producers in Manitoba. The development of white mould during each growing season depends on the moisture and temperature conditions leading up to and during flowering. Under yield-limiting disease pressure, farmers rely on fungicide to control this disease.

Different products are currently available for control of white mould in dry beans. The objective of this study was to evaluate the effectiveness of different fungicide options with a range of active ingredients in pinto beans across multiple growing seasons in Manitoba.

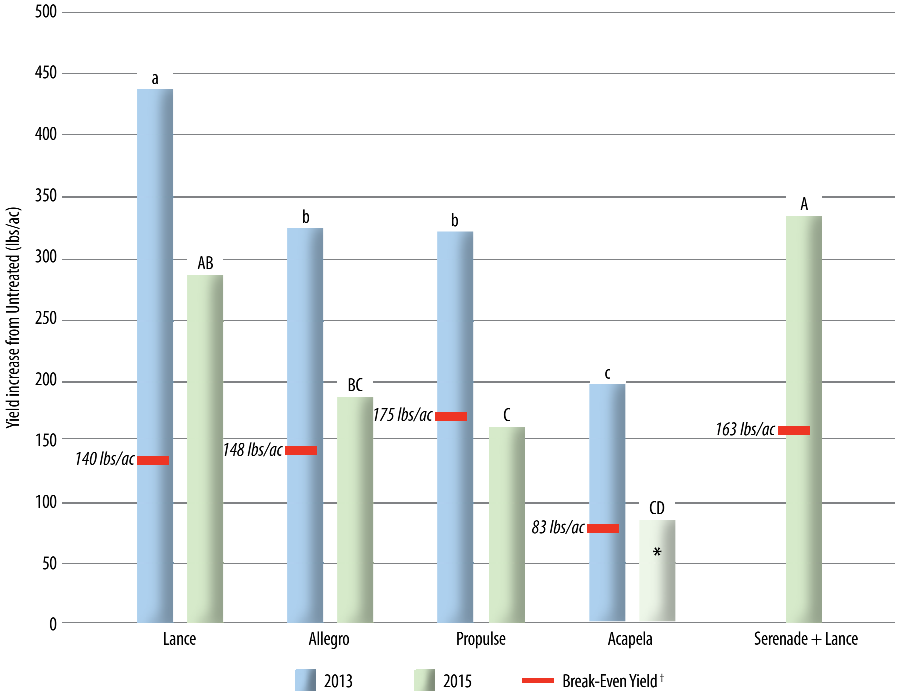
Replicated and randomized full-scale field trials were conducted at Carman from 2013 to 2015. Fungicides included various combinations of Allegro, Acapela, Lance, Propulse and Serenade compared to an untreated check (Figure 1). All fungicide treatments were applied according to label directions, mainly at the R2 (early pin bean) stage. Trials were undercut and picked up in four pairs at the time of harvest.

Significant yield differences among fungicide treatments occurred in two out of three years. This is due to higher white mould pressure in 2013 and 2015, compared to low disease pressure in 2014. In 2013, all fungicide treatments produced higher yields than the untreated check, but Lance outperformed the other fungicides. In 2015, all fungicide products increased yield over the untreated check except for Acapela (Figure 1). Yields were similar among some fungicide products in 2015. However, Lance and Lance + Serenade generally outperformed Allegro, Propulse and Acapela.

An economic analysis was conducted on the responsive years using suggested retail prices (SRPs) for each fungicide, an application cost of $7/acre and a pinto bean price of $0.30/lb. Based on the results of this study, the yield increase required to break even (red bar) ranged from 83 lbs/ac for Acapela to 175 lbs/ac for Propulse (Figure 1).

The results of this study have shown that fungicide efficacy differs among products and is influenced by growing season conditions. In most cases, each product resulted in higher yields compared to the untreated check when white mould pressure was high. Overall, the return on investment will depend on anticipated white mould pressure, the cost of each fungicide product and the yield response from each product. ◗

Figure 1. Pinto bean yield increases (lbs/ac) from different fungicide products and the break-even yield for each fungicide (2013, 2015).



Different letters within a year denote statistically significant differences between products. * No significant difference between treated or untreated.

† Economic analysis performed with suggested retail prices for each fungicide, application cost of $7/ac, and pinto bean price of 30 cents/lb.

PRINCIPAL INVESTIGATOR Brent VanKoughnet, Agri-Skills Inc.

MPSG INVESTMENT $62,100

DURATION 3 years

on-farm network
PARTICIPATORY • PRECISE • PROACTIVE

Enhancing Water Stress Tolerance in Soybeans Through Phytoglobin Manipulations

Phytoglobin levels can be used as a rapid screen to select soybean varieties that are tolerant to excess moisture and drought stress.

SOYBEANS GROWN IN Manitoba may be subject to flooding and/or drought in any given year. With the high proportion of clay soils across the province, water levels tend to recede slowly and can inflict yield loss if excess moisture is prolonged. However, soybeans also require 16–20 inches of water throughout the growing season and can suffer if there is insufficient moisture, especially from late July through August when they need it most.

Having soybean varieties that could tolerate both flooding and drought stress would be a real advantage in Manitoba. Research on plant proteins called phytoglobins (Pgbs) and their role in plant tolerance to environmental stressors may be one way to achieve this.

Pgbs are like animal hemoglobins that bind to oxygen and remove nitric oxide, which damages plant cells and tissue if left to accumulate. Nitric oxide build-up is a plant response linked to both flood and drought conditions. This suggests the potential for Pgbs to improve tolerance to both types of water stress.

The goals of this research were to:

1. screen commercially available soybean varieties for their ability to tolerate water stress and correlate their ability to Pgb level, and
2. assess water stress tolerance of transgenic plants that have experimentally increased or decreased Pgb levels.

In this study, the behaviour of 20 commercial soybean varieties to submergence and waterlogging was characterized. This has helped establish the relationship between Pgb expression level and tolerance to water stress. Natural variations of Pgb can be exploited to select varieties that cope with both types of stress.

Transgenic soybean varieties were successfully developed that either over-produce or under-produce Pgb. From this work, we can confirm that Pgb levels directly correlate with the soybean plant's ability to recover from excess moisture.

Overproduction of Pgb is desirable for improved plant tolerance to water stress. Plants that overproduced Pgb grew better, had a higher photosynthesis rate and produced more adventitious roots — all signs of recovery from moisture stress (Figure 1). Preliminary research has shown that seed number per plant is also enhanced by the overproduction of Pgb.

In addition to recovery from stress, high levels of Pgb in root tissues help protect cells from dying under moisture stress. Plants with high Pgb can then initiate more root growth to recover, compared to plants with low Pgb that slow their root growth in response to stress.

Further studies were initiated at the end of this project to test the potential of using phytoglobin as a powerful molecular marker to select soybean varieties that are tolerant to flooding and waterlogging.

Through either traditional plant breeding techniques or modern gene engineering and editing, the results of this work can be applied to improve flooding and/or drought tolerance in commercial soybean production in the Canadian prairies.

Figure 1. Soybean plant recovery after submergence showing tolerant (left) and susceptible plants (right).

PRINCIPAL INVESTIGATOR Claudio Stasolla, University of Manitoba
MPSG INVESTMENT $96,000
CO-FUNDERS Mitacs, Grain Farmers of Ontario (GFO)
DURATION 3 years

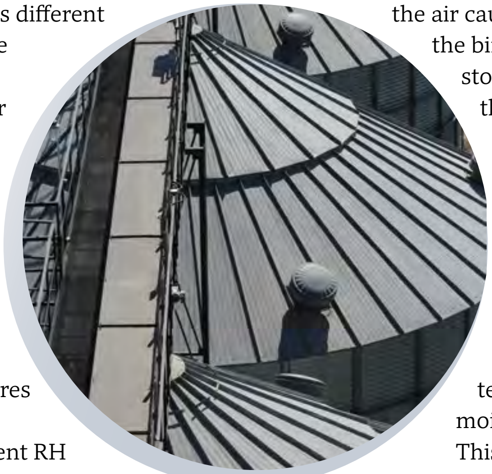
Equilibrium Moisture Content and Safe Storage Guidelines for Soybeans

Soybeans must be stored at temperatures less than 10°C to reduce relative humidity in the bin and minimize spoilage.

SOYBEANS MUST BE stored at 13% seed moisture to avoid spoilage during storage. However, like other crops, stored soybeans reach equilibrium under whatever temperature and humidity conditions exist in the bin. The moisture content at which grain will settle if the air temperature and humidity remain constant for a length of time is referred to as the equilibrium moisture content (EMC).

Tables of EMC values have been calculated for most crops in order to predict the grain moisture content at various temperature–humidity conditions. Soybean farmers should take particular care to avoid exposing soybeans to high temperature and relative humidity (RH) conditions inside the bin. This is because the EMC reached under these conditions will result in biochemical changes that reduce the quality of the crop. To date, EMC's for soybeans produced and stored under typical Manitoba conditions have not been calculated.

Three soybean varieties with a wide range in seed size were selected for this study. To encompass different post-harvest storage conditions, these varieties were either freshly harvested, subjected to three drying and wetting cycles or subjected to three freezing and thawing cycles. They were also subjected to six different temperatures ranging from 5°C to 30°C and five different RH values for a typical range in storage humidity.



This study produced EMC's that, once in table form, will help predict the final moisture content of soybeans exposed to air that is at a specific temperature and RH.

The results of this study have shown that it is critical to keep storage temperatures low (<10°C) for soybeans at any given seed moisture content. At higher temperatures, soybeans equilibrate with the air causing high RH inside the bin (Figure 1). Warm storage conditions in the bin could generate high moisture air pockets due to condensation, triggering mould growth. This highlights the importance of monitoring bin temperature and moisture conditions.

This research has provided a basic tool for predicting the safe storage guidelines of soybean varieties grown in a Manitoba environment. By storing soybeans correctly, farmers can maintain quality, prevent losses and ensure a marketable crop.

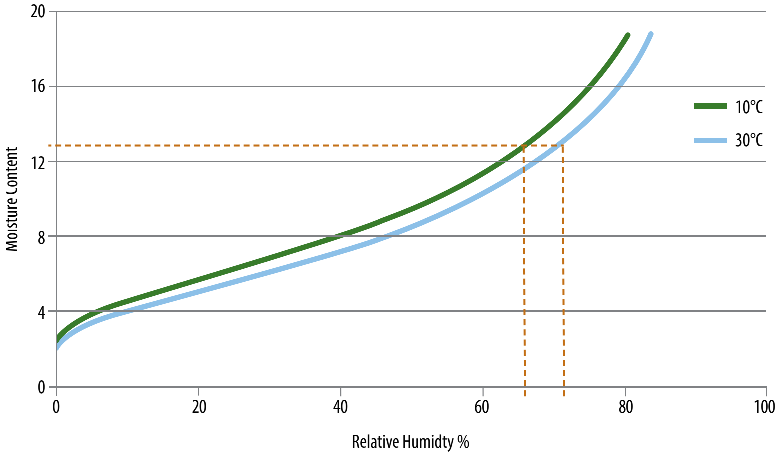


Figure 1. Comparison of the relative humidity of soybeans at 13% moisture content stored at 10°C and 30°C. Storage at high temperature results in higher relative humidity, which should be avoided to prevent soybeans from spoilage.

PRINCIPAL INVESTIGATOR Jitendra Paliwal, University of Manitoba
CO-FUNDER Mitacs
MPSG INVESTMENT $32,743
DURATION 1 year

Air Seeder Distribution and Seed Damage to Soybeans, Wheat and Canola

Higher seed moisture and lower fan speed can reduce the damage inflicted on soybean seed in an air seeding system.

WE HAVE SEEN an increase in the size of farm equipment over time for improved efficiency. In the case of 60-ft air seeders, this raises concerns over how uniformly crops can be seeded and the risk of seed damage. The wider the air seeder, the greater the airflow required to carry seeds down the length of the implement and to prevent plugging. However, excessive airflow can cause seeds to bounce and result in damage by moving seeds more aggressively through the unit.

Large-seeded crops like soybeans are at higher risk of splitting and seed coat damage. This type of damage increases seed mortality and reduces germination. Air seeders and planters are both viable options for planting soybeans in Manitoba, but information is currently limited on how damage occurs in an air seeder and what impact seed moisture content might have on this damage.

The goal of this study was to understand the consistency of distribution in large air seeders (60-ft wide Bourgault and John Deere) and the effect of fan speed on distribution and damage to large (soybeans), medium (wheat) and small (canola) seeds. Three fan speeds were tested for each seed type, including the lowest setting recommended by the manufacturer (low speed), 15% higher RPM (medium speed) and up to 30% higher RPM (high speed). These speeds were tested on soybean seeds at moisture contents of 8%, 10% and 13% at the time of seeding.

Seed distribution was acceptable for both air seeders tested. Results suggest that manifolds positioned closer to the centre of the unit were more likely to receive more product than those located at either end of the machine. However, the manifold position had no significant effect on soybean, wheat and canola germination.

Soybean seed damage was minimal for all trials. The influence of fan speed on soybean damage was insignificant. However, seed moisture content had a noticeable effect on germination potential. Soybeans at 13% seed moisture resulted in 3.6% higher germination than those at 8% seed moisture. Fan speed did not affect wheat and canola germination in either system.

A soak test was conducted on soybean samples collected from the John Deere air seeder openers to assess the damage inflicted on the seed coat. Fan speed and manifold position had no statistically significant impact on seed quality. However, soybean seeds at 8% moisture had more seed coat damage than those at 10% and 13%.

Overall, a combination of lower fan speed and higher seed moisture content presents the best opportunity for reducing soybean seed damage in an air seeding system. It is important to note that each seeder used in this study has a unique distribution system and results gained from one cannot be applied to another. This highlights the need to test these results on individual farms, for different seeding systems and different seed lots. Assessing plant stand each spring is one way to keep track of seed survivability, which pencils out into profitability. ◗

Bags were placed around each opener to collect product during each repetition.

PRINCIPAL INVESTIGATOR Lorne Greiger, Prairie Agricultural Machinery Institute (PAMI)

MPSG INVESTMENT $45,360

CO-FUNDERS Manitoba Wheat and Barley Growers Association, Manitoba Canola Growers Association

DURATION 1 year

The Influence of Soybean Frequency in Rotation and the Persistence of Rhizobia in Manitoba Soils

Bradyrhizobium japonicum persists in Manitoba soils over winter and for years after inoculation. Crop sequence had a minimal effect on *B. japonicum* populations and the microbial community.

NITROGEN IN SOYBEANS is mainly supplied by a symbiotic relationship between the plant and nitrogen-fixing bacteria, *Bradyrhizobium japonicum*, that lives within soybean nodules. These N-fixing rhizobia strains are not native to Manitoba and inoculation is used to introduce the bacteria into the soil.

At present, very little is known about how crop rotations affect microbial communities and rhizobia populations. The objectives of this research were to quantify how *B. japonicum* overwinters in the soil and to determine how the frequency of soybean in rotation will influence rhizobial and microbial populations. This research was built onto a project conducted by Dr. Yvonne Lawley that examined soybean frequency in rotation.

At Carman, St. Adolphe and Melita, four crop sequences were evaluated — continuous soybean, canola–soybean, corn–soybean and wheat–canola–corn–soybean from 2014 to 2017. In 2017, all four crop sequence treatments were in the soybean test crop phase. Microbiomes and *B. japonicum* levels were evaluated at four time points — before planting, VE, R5 and R8, and were analyzed using 16S rRNA sequencing and qPCR, respectively.

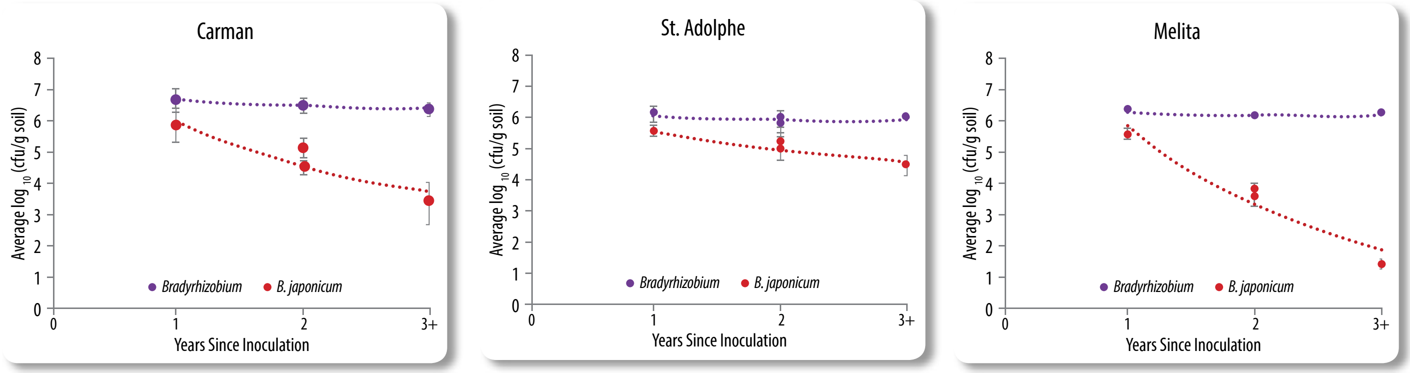
Over winter, the population of symbiotic *B. japonicum* did not significantly decline. Although the rhizobia population persisted in the soil at each site for years after initial inoculation, it declined with time since the last inoculation. The rate of decline was related to the history of soybeans grown in previous years. Fields at Carman and St. Adolphe that had a history of soybeans showed greater persistence of *B. japonicum* over time compared to the first-time soybean field at Melita (Figure 1). The effect of crop sequence on *B. japonicum*, as well as the microbial community, was minimal.

It was also apparent that there are native species of *Bradyrhizobium* present in Manitoba soils that cannot nodulate soybean crops. These non-symbiotic rhizobia also responded to the presence of soybeans and increased throughout the growing season.

This research achieved the first in-depth analysis of microbial communities in Manitoba fields and categorized the entire bacterial population of the microbiome at all three sites in 2017. Carman had significantly higher microbial diversity than St. Adolphe and Melita. The principal reason for observed differences between sites seems to be soil properties, specifically soil type and pH. These results have helped provide a better understanding of the complex plant–microbe and microbe–microbe relationships for future research to build on.

Another study led by Dr. Oresnik from 2016 to 2018 researched if we could determine the minimum population of *B. japonicum* that needs to be present in a field to ensure good nodulation. So far, his work has shown a strong correlation between the qPCR assay and predicting nodulation, but has been unable to find the lower limits of the *B. japonicum* population that would require inoculation. Samples are continuing to be analyzed to determine the lower limit and develop this tool for farmers.

Figure 1. Population of Bradyrhizobium *and* B. japonicum *over years since last inoculation. Reported data are from soil samples that were collected prior to planting. Manitoba locations were Carman, St. Adolphe and Melita.*



PRINCIPAL INVESTIGATORS Ivan Oresnik and Patricia Ordonez, University of Manitoba

MPSG INVESTMENT $68,700

DURATION 2 years

The Influence of Soybean Frequency in Rotation on Disease Pressure

The diverse wheat–canola–corn–soy and corn–soy crop sequences resulted in lower root rot severity compared to canola–soy and continuous soybeans.

SOYBEANS HAVE BECOME the third most popular crop grown in Manitoba but are still considered relatively new to the province. On many farms, soybeans are grown frequently throughout a crop rotation. This raises questions about what impact the frequency of soybeans in rotation might have on the risk of disease development.

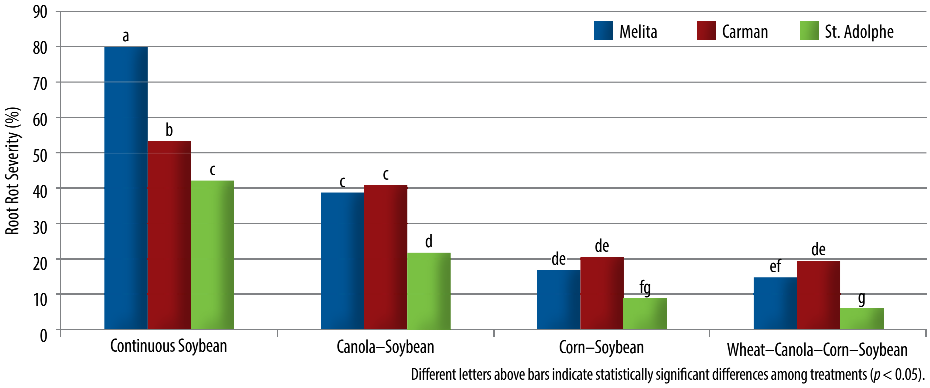
Root and stem pathogens are the most concerning disease pests of soybeans due to their potential impact on yield and quality. Foliar diseases are commonly found but are generally of less concern. According to annual disease surveys, Fusarium root rots have been found in nearly all soybean crops across Manitoba, but often at low incidence. Despite the low incidence of many soybean pathogens to date, disease pressure is expected to rise as soybean production continues.

The goal of this research was to assess the incidence and severity of soybean diseases associated with different crop sequences, building on a project conducted by Dr. Yvonne Lawley examining soybean frequency in rotation.

Lawley's four-year study had four different crop sequences established at Carman, St. Adolphe and Melita from 2014 to 2017: 1) soy–soy–soy–soy, 2) canola–soy–canola–soy, 3) corn–soy–corn–soy and 4) wheat–canola–corn–soy. For the current project, disease pressure was assessed in 2017 when all crop sequences were in the soybean phase (year 4). Ten soybean plants were randomly selected from each plot and brought back to the lab for analysis.

Soybean roots, stems and leaves were visually assessed and given a severity rating. Next, any suspected pathogens were isolated from infected plant parts using conventional laboratory methods (i.e., plated on petri dishes). Finally, diseases were confirmed in the greenhouse following Koch's postulates. This means healthy soybean plants were inoculated with pure cultures of isolated pathogens to confirm the ability of the disease to infect soybeans (i.e., pathogenicity).

Figure 1. Root rot severity (%) in continuous soybean, canola–soy, corn–soy and wheat–canola–corn–soy sequences at Melita, Carman and St. Adolphe in 2017.



Different letters above bars indicate statistically significant differences among treatments ($p < 0.05$).

Koch's Postulates

In 1890, the German physician and bacteriologist Robert Koch established four criteria for assessing whether a given pathogen is the cause of a disease:

1. **the pathogen must be present in all cases of the disease,**
2. **it can be isolated from the host and grown in pure culture,**
3. **it is reproduced when inoculated into a healthy host and**
4. **it is the same as its original form when recovered from the infected host.**

This research identified several soybean diseases, including root rot, anthracnose (*Colletotrichum truncatum*), white mould (*Sclerotinia sclerotiorum*), pod and stem blight/Phomopsis seed decay (*Diaporth/Phomopsis* complex) and frogeye leaf spot (*Cercospora sojina*). Fusarium root rot was the most prevalent and damaging disease (Figure 1). Of the seven total *Fusarium* species identified, *F. oxysporum* was most common, making up 50% of the isolates.

Additional organisms were found, including *Colletotricum spaethianum* which is not known to infect soybeans and *Plectosphaerella cucumerina*, a potential latent infection that may live its life in the plant without expressing visual symptoms.

The diverse wheat–canola–corn–soy and corn–soy crop sequences resulted in the lowest root rot severity. However, alternating canola–soy had greater root rot severity, following a similar trend as continuous soybeans. These results suggest that root rot pressure is more variable and generally riskier when alternating soybeans with another crop, compared to growing soybeans in a more diverse rotation.

Root rot severity also varied by location. Continuous soybeans resulted in greater root rot severity at Melita (80%) compared to Carman (53%) and St. Adolphe (42%) (Figure 1). This highlights the need to assess disease risk on a field by field basis, rather than by crop sequence alone.

PRINCIPAL INVESTIGATOR Fouad Daayf, University of Manitoba

MPSG INVESTMENT $106,000

DURATION 2 years

The Most Comprehensive Survey of Soybean Foliar Diseases in Manitoba

Frogeye leaf spot and bacterial pustule were identified for the first time in Manitoba in 2016 using molecular analyses.

EACH YEAR, A range of foliar diseases are present in soybean crops across Manitoba. Timely and accurate assessment of these pathogens is important to determine the impact on production and what type of management is appropriate.

Traditional disease surveillance has relied on approaches that only validate the presence of known foliar diseases. This suggests a need for tools and techniques that identify any emerging diseases.

The objectives of this study were to: 1) identify and assess the prevalence of known and emerging soybean foliar diseases, 2) evaluate the incidence of co-infections to characterize pathogens that may act synergistically and 3) quantify genetic diversity among strains/races of pathogens.

Soybean fields were surveyed for foliar diseases in 2016 (81 fields) and 2017 (67 fields), in collaboration with MPSG and Manitoba Agriculture. At least five leaves showing foliar disease-like symptoms were collected per field at the V3 and R6 stages.

Each leaf was preserved in a special solvent and brought back to the lab for next-generation sequencing (NGS). NGS is a relatively new and powerful disease diagnostic tool that can identify both new and existing diseases in Manitoba. This generated DNA sequence data for pathogen strains specific to Manitoba, which was used to develop molecular diagnostic tools for seven of the most common foliar diseases. These diagnostics will improve the accuracy of disease identification in future surveys at a minimal cost.

Two diseases were identified for the first time in Manitoba, including frogeye leaf spot (*Cercospora sojina*) and bacterial pustule (*Xanthomonas spp.*). Residual diseases were also identified, meaning pathogens present in the previous year's crop were still active in soybeans grown the following year. This generated important knowledge for crop rotation planning.

This project helped detect pathogens that are new to a region and those not yet on the radar of agronomists. Along the way, it also validated visual disease diagnoses made in the field. While most of these foliar diseases are not considered economically relevant, prevalence (% of total fields infected) and incidence (% of plants infected within a field) should be monitored over time and managed accordingly.

Photos and descriptions of all diseased leaves were compiled and made available at manitobapulse.ca. ◗

Table 1. Foliar pathogens identified by molecular analyses at early (V3) and late (R6) survey timings in the western, central and eastern regions of Manitoba (2016–2017).

🟩 Disease present ⬜ Disease not present ▨ n/a

		Western				Central				Eastern			
		2016		2017		2016		2017		2016		2017	
Type	**Pathogen**	V3	R6	V3	R6	V3	R6	V3	R6	V3	R6	V3	R6
Fungi	Leaf spot (*Alternaria* spp.)	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	🟩	🟩
	Leaf blight (*Cercospora kikuchii*)	⬜	⬜	⬜	⬜	🟩	🟩	🟩	🟩	▨	🟩	⬜	⬜
	Frogeye leaf spot (*Cercospora sojina*)	🟩	🟩	⬜	⬜	🟩	🟩	🟩	🟩	▨	🟩	🟩	🟩
	Anthracnose (*Colletotrichum* spp.)	⬜	⬜	🟩	🟩	⬜	🟩	🟩	🟩	▨	⬜	🟩	🟩
	Ascochyta blight (*Didymella pinodes*)	⬜	⬜	🟩	🟩	⬜	⬜	🟩	🟩	▨	⬜	🟩	🟩
	Leaf spot (*Drechslera* spp.)	⬜	⬜	🟩	🟩	⬜	⬜	🟩	🟩	▨	⬜	🟩	🟩
	Downy mildew (*Peronospora manshurica*)	⬜	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	🟩	🟩
	Leaf blight (*Pleospora herbareum*)	⬜	⬜	🟩	🟩	🟩	⬜	🟩	🟩	▨	⬜	⬜	⬜
	Brown spot (*Septoria glycines*)	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	🟩	🟩
Bacteria	Bacterial blight (*Pseudomonas syringae*)	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	🟩	🟩
	Bacterial pustule (*Xanthomonas* spp.)	🟩	⬜	⬜	⬜	🟩	🟩	⬜	⬜	▨	⬜	⬜	⬜
Virus	Necrotic spots (*Tobacco necrosis virus*)	⬜	🟩	🟩	🟩	⬜	🟩	⬜	⬜	▨	🟩	🟩	🟩
	Alfalfa mosaic (*Alfalfa mosaic virus*)	⬜	⬜	🟩	🟩	⬜	⬜	🟩	🟩	▨	⬜	🟩	🟩
	Viral infection (*Bean yellow mosaic virus*)	⬜	🟩	⬜	⬜	⬜	⬜	⬜	⬜	▨	⬜	⬜	⬜

PRINCIPAL INVESTIGATOR Bryan Cassone, Brandon University

MPSG INVESTMENT $112,509

DURATION 2 years

Phytoferritin: A Potential Iron Supplement Derived from Manitoba Pulses and Soybeans

Phytoferritin derived from legumes is a readily available and quickly digestible source of iron. From this research, an extraction and isolation protocol compatible with industrial, large-scale production was developed.

IRON DEFICIENCY AFFECTS approximately 3.5 billion people worldwide. Iron deficiency can result in anemia, which is a reduction in the number of red blood cells and hemoglobin in the blood, leading to an insufficient oxygen supply to muscles and vital organs.

Iron-containing supplements are used to boost the availability of dietary iron. Most current supplements have been associated with negative symptoms, resulting in lower supplement consumption. Alternatively, certain plant sources of iron can help improve consumption. Plant-based iron boosts plasma iron levels in the blood, preventing iron deficiency anemia and other negative symptoms.

Phytoferritin is a well-known organic form of iron that can be isolated from plants, especially pulse and soybean seeds, for use as an ingredient in nutraceutical supplements. Phytoferritin is essentially a storage molecule that holds up to 4500 iron atoms within its core, while other sources average around 2500 iron atoms.

Based on previous research, the low dose of ~2.4 mg phytoferritin/day could be used to improve iron deficiency symptoms. Thus far, clinical trials have shown that phytoferritin does not produce the same intense negative symptoms as traditional iron supplements. Nor does it suffer from low bioavailability.

In general, pulse and soybean seeds have higher levels of iron and phytoferritin than cereal grains, attributed in part to the large iron requirement during nitrogen fixation by nodules. Phytoferritin is recycled to the seed during nodule breakdown. Concentrations can vary between 8–80 micrograms of phytoferritin per gram of seed, with the highest concentrations in peas, soybeans and mung beans.

This research aimed to establish Manitoba-grown pulse and soybean crops as important sources of phytoferritin. To date, it has provided information on extractability, as well as structural and functional components, adding value to our Manitoba pulse and soybean crops.

The first objective of this research was to determine the yield and purity of phytoferritin concentrates isolated from soybeans, yellow peas, red and white dry beans, lentils, mung beans and chickpeas. As a result, an extraction and isolation protocol compatible with industrial-scale production was developed to produce phytoferritins from Manitoba legume seeds. Phytoferritin yield ranged from 7% to 19% (seed weight basis) with an iron content of up to 45 mg/100 g weight.

The second objective was to determine the susceptibility of phytoferritin to breakdown by gastrointestinal enzymes through simulated stomach and intestinal digestion treatments. Phytoferritins first subjected to pepsin, a main digestive enzyme to simulate stomach breakdown, followed by another enzyme pancreatin to simulate intestinal breakdown, were successfully digested.

Phytoferritin digestion led to the complete release of iron, indicating that the product is compatible with the human digestive tract. During digestion, iron release occurred very quickly and was completed within 45 minutes. Therefore, it is very likely that the phytoferritin iron load will empty very quickly within the upper tract of the digestive system, which is excellent for absorption into the blood circulatory system.

This research has successfully demonstrated that phytoferritins derived from pulse and soybean seeds are digestible. It has also resulted in protocols that are compatible with industrial production to produce plant-based iron supplements, benefitting the pulse and soybean value chain. ◗

PRINCIPAL INVESTIGATOR Rotimi Aluko, University of Manitoba
MPSG INVESTMENT $5,000
CO-FUNDER Agri-Food Research and Development Initiative (ARDI)
DURATION 2 years

The Effect of Genotype and Environment on Pulse Flour Quality and Baking Performance

Bean variety, growing location and crop year can influence the quality and sensory characteristics of bread baked from bean flour.

Bread made with 20% black bean flour and 80% wheat flour (left) compared to bread made with 100% wheat flour (right).

CONSUMER DEMAND FOR healthier and more innovative bakery goods continues to grow. This represents a large market opportunity for the use of pulse flours in baking applications. Flours made from pulses not only enhance the nutritional properties of bread, but they also offer a range of functionality and flavour profiles.

Bean flours have been proven to function well in numerous baking applications, including bread. Understanding the effects of varieties and growing locations on the compositional, functional and flavour properties of pulse flours is critical for expanding the market for bean flours as alternative plant-based protein ingredients. This can also help minimize any lot-to-lot variation in pulse flour quality.

The objective of this research was to examine the effects of bean variety (i.e., genotype), growing location and crop year (i.e., environment) on the compositional and functional characteristics of flour milled from four market classes of beans — navy, pinto, black and cranberry. This study also examined the effect of variety and growing location on baking performance and flavour properties of bread.

Different bean varieties were grown at various locations in Manitoba from 2016 to 2017. The beans were milled into flour and analyzed for their protein and starch contents, pasting properties, water absorption capacity and particle size properties. Pulse flours were blended with wheat flour (20:80/pulse:wheat) and baked into bread (pictured above). Bread quality parameters (e.g., colour, specific volume and C-cell properties) were measured and sensory characteristics (e.g., appearance, aroma, pulse flavour, sweetness, bitterness, after taste and overall acceptability) were analyzed using a trained sensory panel.

Results revealed that variety, growing location and crop year significantly affected the properties of pulse flour. Bread quality and sensory properties were also significantly affected by a combination of these three variables. These results are of practical importance to flour millers and bakers in selecting specific bean varieties with the desired quality traits for bakery applications.

Knowledge gained from this research is beneficial to the entire pulse value-chain, including breeders, seed companies, producers, pulse ingredient processors and the food industry. These findings will allow breeders to target specific end-use quality characteristics as part of their breeding program, leading to variety development that meets marketplace demands. Pulse processors may also be able to control some of this variability by sourcing specific varieties from producers. ◗

PRINCIPAL INVESTIGATOR Elaine Sopiwnyk, Canadian International Grains Institute (Cigi)

MPSG INVESTMENT $25,000

CO-FUNDERS Western Grains Research Foundation (WGRF), Agri-Food Research and Development Initiative (ARDI), Saskatchewan Pulse Growers (SPG)

DURATION 2 years

Processing of Soybeans to Improve Palatability and Digestibility of Soy-Based Foods

Infrared heat treatment (micronization) of intact soybean seed can effectively reduce the undesirable beany flavour and trypsin inhibitor levels in soy-based food products, improving palatability and digestibility.

PRODUCTS MADE FROM soybean meal have been linked to reduced risks of cancer, osteoporosis, renal disease and heart disease. These health benefits may be attributed mainly to soy protein. Despite these benefits, there are some limitations to soy-based products such as a beany flavour that consumers deem undesirable and trypsin inhibitors that interfere with the ability of trypsin to digest protein in the diet.

The beany flavour has been linked to volatile flavour components in soy products. These volatile components result from the oxidation of fatty acids by the enzyme lipoxygenase. Different approaches have been examined to reduce these volatiles, including heat treatment. However, heat can change the way soybean protein behaves in food products. For example, it can reduce the level of protein in soymilk.

The objective of this study was to reduce the undesirable beany flavour and trypsin inhibitor levels in soybeans without affecting protein quality and its functioning in food products. A variety of treatments, such as micronization (infrared heating), ethanol extraction and treatment with the enzyme alcalase, were used alone or in combination to test this.

Micronization treatments (no heat, 100°C heat and 135°C heat) were used on whole soybean seeds at 13% moisture content. The seeds were then de-hulled and milled into flour. The flour was defatted with hexane to mimic industrial oil removal and the fat content was reduced from 25% to below 3%. Three ethanol treatments (0, 65% or 85% ethanol washes) were then applied to remove the lipid component further. The final treatment on the flours was hydrolysis with the alcalase enzyme, which breaks down protein.

Micronization alone effectively reduced trypsin inhibitor activity (TIA). TIA was reduced by 50% at 100°C and reduced by 80% at 135°C. In most cases, the ethanol treatment did not reduce TIA. It actually increased TIA in non-micronized soybean flour samples. Alcalase treatment results were more variable. Alcalase reduced TIA in the non-micronized soy flour samples, had no effect on the samples micronized to 100°C and those treated with ethanol, and increased TIA in flour samples that were pre-treated with heat at 135°C. While alcalase was able to reduce TIA in unheated samples, there was no benefit when combined with a heat treatment.

Lipoxygenase (LOX) activity, related to the undesirable beany flavour, was very sensitive to heat. After micronization at 135°C, 99.6% of the LOX was inactivated. In addition, LOX activity was not found in samples treated with ethanol or alcalase. This means that all three methods show promise for reducing the volatiles that cause the beany flavour.

Overall, a moist heat treatment like micronization applied to intact soybean seed is the most effective way to reduce both beany flavour and trypsin inhibitor levels in soy-based food products. Improved palatability and digestibility of these products will then increase the potential for uptake and demand. The next step of this research would be a sensory study to test these findings on consumer perception of beany flavour in soy products. ◗

PRINCIPAL INVESTIGATOR Susan Arntfield, University of Manitoba

MPSG INVESTMENT $46,000

DURATION 2 years

Enhancing World Markets for Canadian Pulses: Quality and Functionality of Pulse Ingredients

Technical information on pulse ingredients has been communicated to key members of the value chain to demonstrate the quality and functionality of pulses in food products as a result of this research.

PLANT-BASED PROTEINS ARE gaining momentum in the food industry, with growing interest specifically in pulse ingredients. Pulse ingredients derived from crops like peas, dry beans and lentils are low-allergenic and have potential to increase the marketability of plant-based food products. Providing information on the quality and functionality of Canadian pulses can enhance Canada's image as a supplier and improve subsequent demand for our pulse crops.

A series of market-responsive applied research experiments were initiated by the Canadian International Grains Institute (Cigi) to explore the quality, processing and utilization of pulse ingredients. The goal was to communicate the findings of these experiments to key members in the pulse value chain for enhanced marketability opportunities.

Several achievements were made by Cigi throughout the course of this study to advance marketing opportunities for Canadian pulse ingredients. Firstly, research on the standardization of pulse flours has helped several food companies gain a better understanding of pulse ingredients. This means they can incorporate pulses while maintaining the uniformity and consistency of their products.

Other pre-commercialization work demonstrated the successful incorporation of pulse flours into high-quality puffed (i.e., extruded) snacks, crackers and pasta. They found that pulse flours increased protein and total dietary fibre of these foods while maintaining an appealing colour and flavour. In one specific case, Cigi provided these results to a food company and connected them with a Canadian pulse ingredient supplier to increase the amount of Canadian lentil flour in their formulation, while meeting their product needs.

Pre-milling seed treatments, such as micronization (i.e., infrared heating) and partial germination of pulses, were examined to harness any additional benefits of pulse ingredients. Cigi examined the effects of these treatments on the quality and functionality of end products like pasta and snacks. They found that micronization improved/brightened spaghetti colour but changed certain functional characteristics (e.g., pasting profile, emulsifying capacity). Partial germination of peas had an overall minimal impact on the texture of spaghetti. These findings have since been shared with food processors.

Cigi has also participated in several investigative missions to assess the opportunities for Canadian pulses as ingredients in international markets. Numerous contacts have been made with food processors in Mexico, who are now interested in adding Canadian pulses to their products.

Technical information can be drawn from this research to support value-added initiatives like secondary processing, support plant breeding efforts and promote Canadian pulses in domestic and international markets. For more details on the experiments conducted within this study, visit manitobapulse.ca.

Ongoing research and future goals include a focus on pulses and pulse ingredients in the gluten-free processed food industry. This work is focused on creating nutritionally improved, high-quality gluten-free food products that are desirable to both consumers and manufacturers. ◗

Dried untreated pulse flour spaghetti (top) compared to micronized pulse flour spaghetti (bottom) and a semolina control (centre). Left to right: Spaghetti made with yellow pea, green lentil, red lentil, chickpea and navy bean flour.

PRINCIPAL INVESTIGATOR Linda Malcolmson, Canadian International Grains Institute (Cigi)

MPSG INVESTMENT $43,000

CO-FUNDERS Saskatchewan Pulse Growers (SPG), Agriculture and Agri-Food Canada (AAFC) – Agricultural Innovation Program

DURATION 5 years

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