

MANITOBA PULSE & SOYBEAN GROWERS

pulsebeat

The Science Edition

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Yield & Quality • Soil Health • Pest Control
• Market Demand

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

Thank you for taking the time to read the latest edition of *Pulse Beat: The Science Edition*. For all the time we at Manitoba Pulse & Soybean Growers (MPSG) spend on planning and negotiating research projects, we take the most pride in being able to communicate the results to you, the farmer, through mediums such as this magazine.

Maybe it's the breadth of information generated by the projects – this edition's pages speak to everything from optimum planting and weed-free windows for soybeans to how much salt chefs should add to achieve the perfectly cooked bean.

Or, maybe it's the satisfaction of knitting together results from unrelated studies – the On-Farm Network asked if fields with a history of soybeans should continue to be inoculated while, elsewhere, a more complex genomic study revealed the extent to which even those well-inoculated soils can harbour *Fusarium* root rot.

Whatever ignites our desire to communicate, we know the topics of interest to pulse and soybean growers are many and varied. Therein lies our challenge.

Being responsive to farmers' needs means research can fly in many directions. Sometimes the sheer variety of results can require time to put together a practical message that is useful to farmers. For instance, we see in this edition light being shed on soybean protein – certainly, a topic of interest. Don't expect a hard and fast recommendation, though. There's more to figure out.

So, amidst the gush of research on complex issues, we will always seek the coherent message that ties it together to benefit our farmers. We continue to hang this effort on the MPSG theme: profit and sustainability arises from knowledge that enables farmers to 1) improve yield, 2) meet market quality while 3) economically managing pests and 4) continually building their soil.



Daryl Domitruk
MPSG Director of Research and Production
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On-Farm Evaluation of Single versus No Inoculation of Soybeans

There was no yield response to single inoculation on fields with a history of at least three, well-nodulated soybean crops with the most recent soybean crop grown in the past four years.

SOYBEANS ACQUIRE BETWEEN 46–74% of their nitrogen (N) through biological N fixation in root nodules. This process takes place due to a symbiotic relationship between the soybean plant and *Bradyrhizobium japonicum*, the bacteria included in soybean inoculant products.

In previous on-farm studies, MPSG has found limited soybean yield response to double inoculation (two formulations or placements of inoculant), compared with single inoculation on fields with at least two years of soybean history. This prompted the question: would inoculation still be necessary when there is a more extensive history of well-nodulated soybean crops?

The objective of this on-farm trial was to quantify the agronomic and economic impacts of seed-applied inoculant versus no inoculant in soybean fields with a history of at least three previous, well-nodulated soybean crops.

Twenty-seven trials were established on soybean production fields in the central, eastern and Interlake regions of Manitoba from 2016 to 2018. Fields had a history of at least three well-nodulated soybean crops and the most recent soybean crop was established in the past four years. Crop rotation, soil characteristics, soil fertility and field equipment varied between trial sites covering a broad range of field characteristics.

There was no significant yield response to a seed-applied, single inoculation treatment compared to an untreated, uninoculated check. Soybean plants were evaluated at R1 (beginning bloom) for nodulation. All plants examined were found to have adequate nodulation, having at least 10 nodules per plant. No visual differences between treatments nor signs of N deficiency were observed.

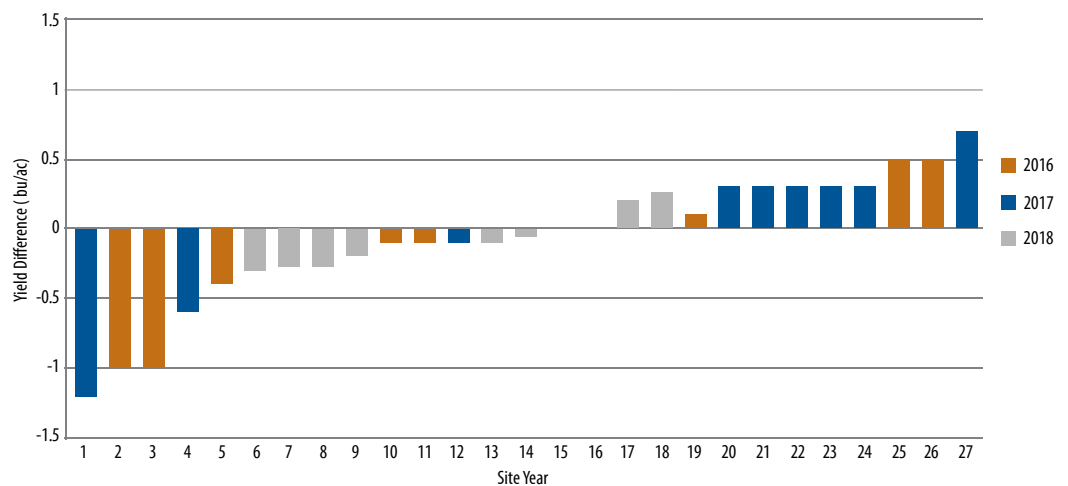
These research results suggest that soybean inoculation is not needed when

there is a history of at least three well-nodulated soybean crops on a field where soybeans had been grown in the last four years. However, some may choose to continue to utilize a single inoculation strategy on their farm as the cost of a single application of liquid inoculant on seed is relatively inexpensive compared to the risk of a non-nodulated soybean crop.

Factors that may reduce survival of inoculum in the soil should be considered before eliminating inoculation. Risk factors include non-optimal soil pH (less than five or above eight), soils with a high percentage of sand and prolonged water stress (flooding or drought). There are currently no tools available to farmers that estimate residual soil inoculum levels.

A related MPSG-funded research project led by Dr. Ivan Oresnik, at the University of Manitoba, evaluated soil samples taken from these treatments to quantify the population of *B. japonicum* in the soil. The results from this study will contribute to the development of a rapid soil test to determine the levels of *B. japonicum* within a field. With the ability to test bacterial levels, a recommendation could be given on whether the population in the soil is sufficient for good nodulation and whether inoculation would be profitable. ■

Figure 1. Soybean yield difference between single and no inoculation at 27 sites from 2016 to 2018. No significant differences were present between sites.



PRINCIPAL INVESTIGATORS Manitoba Pulse & Soybean Growers and Tone Ag Consulting

MPSG INVESTMENT \$123,000

DURATION 3 years



Assessing Soybean Inoculant Strategies

Inoculant increased yield, nodule number and seed protein compared to uninoculated soybeans on fields with no history of soybean. Inoculant products, formulations, rates and combinations performed similarly, regardless of field history.

SELECTING AN APPROPRIATE soybean inoculant is dependent on field history, equipment availability, inoculant cost and environmental conditions. Seed-applied liquid- and peat-based products are cheap and conveniently applied to the seed. In comparison, in-furrow granular inoculant is more expensive and requires an extra seed cart tank to apply, but it is more resilient to environmental extremes. For first- and second-time soybean fields, a “double inoculation” strategy – use of two inoculant formulations or placements (i.e., seed-applied liquid and in-furrow granular) is recommended to ensure adequate rhizobia populations are introduced to the soil. Increasing the rate of inoculant may also achieve the same result, but double inoculation has the potential added benefit of improved rhizobia survivability. Once several successfully nodulated soybean crops have been established over time, farmers may use the more economical single inoculation strategy. Some inoculant

products are formulated to improve early crop development, plant nutrition or the rate of nodulation. Examples of “enhanced” inoculants tested in this study were Jumpstart® and Tagteam® (with phosphate-solubilizing microorganism, *Penicillium bilaii*), Nodulator® N/T (with plant growth promoting rhizobacteria, *Bacillus subtilis*) and Optimize® (with lipochitoooligosaccharide, a signal that initiates nodule development).

This field study was conducted at Melita, Carberry, Carman, Roblin and Beausejour from 2014 to 2016. Fourteen inoculant treatments were compared, including an uninoculated control and different inoculant products (Cell-Tech®, Nodulator®, “enhanced”), formulations (liquid, granular, liquid + granular) and rates (1X, 2X).

Inoculation had important economic implications at the five fields with no history of soybean. On average, inoculant increased the number of nodules per plant by 20, yield by 15 bu/ac and protein by

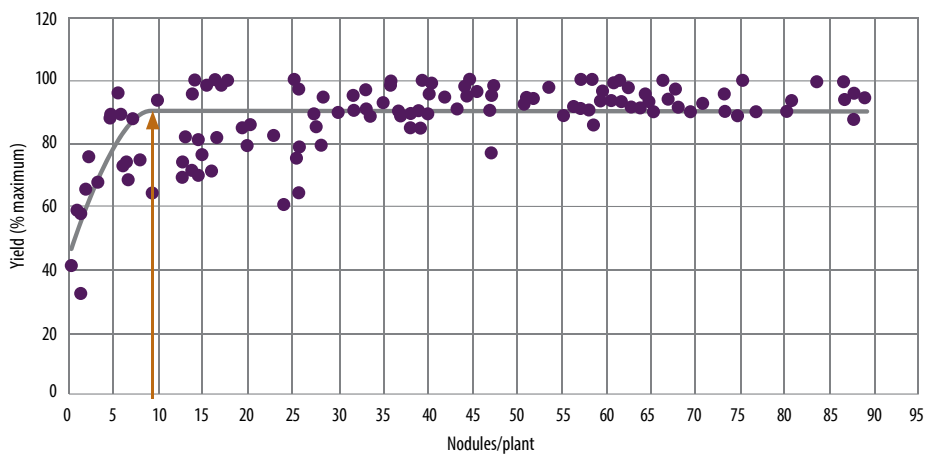
4.8% compared to the uninoculated control. There was no difference in soybean yield, nodulation or protein between individual inoculants, regardless of field history.

Despite the lack of response among inoculant strategies, MPSG still recommends double inoculating fields with a limited history of soybeans and moving to a single inoculation strategy after at least two successfully nodulated soybean crops have been established. There are several possible explanations for the lack of response to double inoculation in this trial, which cannot always be guaranteed under field conditions: 1) soybeans were seeded into ideal soil conditions that were favourable for crop emergence and inoculum survival during late May to early June, 2) inoculants were properly stored, handled and applied, according to label recommendations and 3) there were no inoculant compatibility issues with fungicide and/or insecticide seed treatment, as bare seed was used.

Yield and nodule number were, on average, higher in fields *with* a history of soybeans compared to *no* soybean history (46.4 vs. 39.8 bu/ac, 58 vs. 22 nodules per plant, respectively). However, inoculant had no impact on average seed yield, protein and nodulation in fields *with* a history of soybeans, across four sites.

Regardless of inoculant strategy or field history, nodulation should be assessed in every field, every year. This study found that the minimum number of nodules required to reach 90% of maximum yield was approximately 10 nodules per plant at the R4 stage (Figure 1). Assessing nodulation at R1 allows for rescue nitrogen fertilizer application at the ideal window (R2–R3), ahead of peak soybean N-uptake requirements (R4–R5). ■

Figure 1. Relationship between number of nodules at R4 per soybean plant and relative yield.



PRINCIPAL INVESTIGATOR Manitoba Pulse & Soybean Growers

MPSG INVESTMENT \$17,920 | DURATION 3 years

ACKNOWLEDGEMENT Yvonne Lawley (U of M), Scott Chalmers (WADO), Craig Linde (CMCDC), James Frey (PCDF) and Nirmal Hari (PESAI)

Yield and Maturity of Late-Seeded Soybeans in Manitoba

Soybeans grown in Portage and Morden demonstrated good yield potential and little risk for seeding soybeans as late as June 9 to 12. Seeding between May 31 and June 6 at Arborg reduced yield potential and/or increased risk for not reaching maturity.

NEARLY HALF OF the 95+ soybean varieties evaluated in 2018 fell within the short-season category. These early-maturing varieties require less than 115 frost-free days to reach maturity.

In situations where spring planting is delayed, and farmers are presented with a shorter growing season, could early-maturing varieties be used to achieve acceptable yields and mature before the typical fall frost date?

Soybean seeding deadlines for full insurance coverage are May 30 for Area 2 (Portage), 3 (Arborg, Melita) and 4 (Roblin, Swan River), and June 6 for Area 1 (Morden). These deadlines have not been reviewed since 2005.

This project evaluated the potential of late-seeded soybeans in Manitoba and determined the feasibility of extending current crop insurance deadlines.

From 2015 to 2017, three soybean varieties (very early, early and mid-season) were planted in three seeding windows (late May, early June, mid-June) in Arborg, Portage and Morden. These locations vary in growing season length and latitude, but also represent three distinct Manitoba Agricultural Services Corporation (MASC) insurance areas. To evaluate the potential of late-seeded soybeans, data was collected on plant population, plant height, plant productivity, maturity, yield and seed quality. Regarding decision-making, yield and maturity are the most important variables.

MATURITY

At both Portage site-years, soybeans matured within at least one day of the normal frost date (Sep 25) regardless of seeding date. At Morden in 2017, all soybeans matured prior to the normal frost date (Sep 25), but in 2016, late- and

very late-seeded soybeans matured beyond the normal frost date. As expected, Arborg showed the highest risk associated with seeding soybeans late. Soybeans at Arborg matured five days or more after the normal frost date (Sep 22) when seeded May 31 or later. In addition, two of the varieties at the very late seeding date did not mature in 2016.

YIELD

Soybean yields ranged from 24–53 bu/ac, depending on the site-year. Overall, the very early variety and very late seeding date tended to reduce yield.

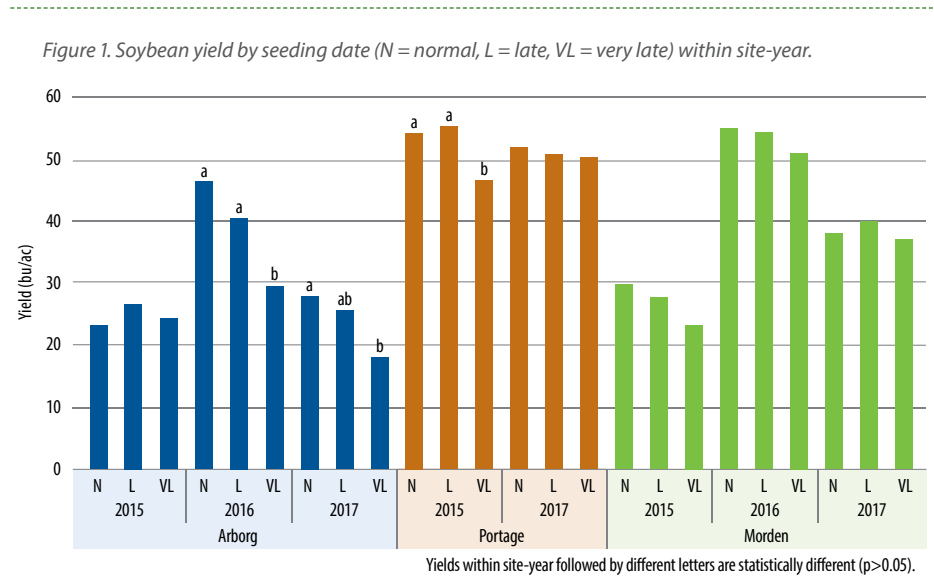
Historically, seeding dates and deadlines have considered 80% yield potential to be an acceptable benchmark. In other words, can late-seeded soybeans maintain 80% yield potential compared to a normal seeding date? To answer this question, the effect of seeding date within site-years was explored (Figure 1).

Soybean yield across seeding dates was statistically similar at most site-years,

except at Arborg, where soybean yield at the very late planting date was reduced to 65–67% of the normal planting date. Yield was reduced due to very late seeding at Portage in 2015, as well, but maintained 84% yield potential compared to the normal seeding date. All seeding dates were delayed at Morden in 2015, which contributed to reduced yields overall.

In summary, based on soybean maturity and yield potential, Portage and Morden site-years demonstrated good yield potential and little risk for seeding soybeans as late as June 12. At Arborg, seeding soybeans beyond June 6 typically resulted in a decline in yield potential and increased risk of not reaching maturity. When soybeans are seeded late, risk may be mitigated with appropriate variety selection.

The results of this research project are being reviewed in consultation with Manitoba Agriculture and MASC to support a review of soybean seeding deadlines for Areas 1–3. ▶



PRINCIPAL INVESTIGATOR Kristen P. MacMillan, University of Manitoba,

MPSG INVESTMENT \$17,610

CO-FUNDERS Growing Forward 2 Growing Innovation: Agri-Food Research and Development Initiative

Evaluating the Effect of Soil Temperature and Planting Date on Soybeans in Manitoba

Delayed soybean planting beyond mid- to late-May had the potential to reduce yields and expose soybeans to fall frost damage. Soil temperatures greater than 10°C did not guarantee improved crop establishment or yields.

COLD SOIL TEMPERATURES at planting and frost in spring and fall pose a risk to soybeans in Manitoba. Planting dates can be manipulated to ensure soybean seeds are planted into the correct soil temperature and to lessen the risk of frost damage in spring and fall. Little is known regarding the impact of planting date and soil temperature on soybean growth, yield and quality under Manitoba conditions.

The objective of this project was to determine the effect of soil temperature at different planting dates on soybean growth, yield and seed quality. Small-plot field trials were conducted from 2014 to 2017 at Brandon, Carberry, Portage la Prairie and Roblin, for a total of 12 site-years.

Soybean planting dates in this study fell into a “recommended” window from mid- to late-May (May 18 to May 29) or “late” window during early June (May 30 to June 11), which was nine to 15 days after the first planting date. Within each planting date category were “cold,” “control” and “warm” soil temperature treatments.

To establish different soil temperature treatments, plots were covered in early spring with foam board/reflective material to insulate the soil (cold), white/clear plastic to reflect the sun (control) or black plastic to warm the soil (warm).

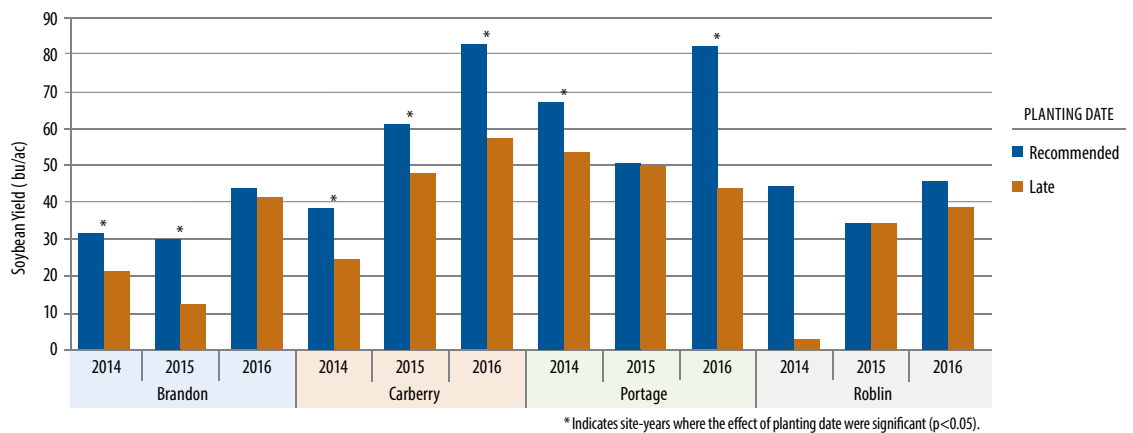
Soybeans planted during early June in this study reached only 40 to 80% of the soybean yields achieved by soybeans planted during mid- to late-May (Figure 1). In one of three years at Roblin, late planting resulted in significant fall frost damage and negligible yields (Figure 1). These findings highlight greater yield potential associated with mid- to late-May planting dates over June planting. They also showcase the risk of soybean yield reduction from fall frost at locations in Manitoba that have shorter growing seasons.

The optimum temperature for soybean germination and emergence is 20 to 22°C, according to previous controlled environment research. In this study, soil coverings produced a range of

soil temperatures under field conditions that were often below 18 to 22°C and occasionally below 10°C. However, soil temperature differences at planting were not consistently associated with differences in soybean yield. This suggests that soil temperature differences among treatments may not have been great enough to affect the soybean crop, or that soybeans were able to compensate for these early-season differences.

Results from this project are supported by another study in Manitoba conducted by Dr. Yvonne Lawley, examining the impact of soil temperatures at planting (6–16°C) on soybean plant establishment and yield. This study also found that calendar date likely had a greater influence on soybean yield than soil temperature at planting, despite a wide range of soil temperatures. In addition, soil temperatures of 14°C or greater at planting resulted in faster soybean emergence, but no differences were found between established plant populations. ▶

Figure 1. Planting date effect on soybean yield averaged across soil temperatures at Brandon, Carberry, Portage and Roblin, Manitoba (2015–2017). “Recommended” planting dates were seeded mid- to late-May and “late” planting dates were seeded early June.



PRINCIPAL INVESTIGATOR Dr. Ramona Mohr, Agriculture and Agri-Food Canada – Brandon

MPSG INVESTMENT \$49,575

CO-FUNDERS Western Grains Research Foundation, Agri-Food Research and Development Initiative

DURATION 4 years

Soybean Performance Under Different Moisture Regimes

Established soybeans are relatively tolerant to excess moisture conditions, but late-season water deficits reduced soybean yields by 16–32%.

SOYBEANS, LIKE ALL crops grown in Manitoba, may be subject to moisture conditions ranging from deficit to excess. Moisture can vary not only among years, regions and fields, but also within a given year, growing region or field. While practices such as irrigation and drainage may be used to manage moisture extremes, it would be advantageous if simpler agronomic practices such as variety selection could be employed to reduce the effects of moisture extremes on crops.

On-farm experience in Manitoba suggests that soybeans are relatively tolerant of excess moisture compared to other commonly-grown crops, while research from the United States suggests that variability exists among soybean varieties in their response to moisture. The aim of this study was to determine if variability existed among soybean varieties with respect to disease, growth and yield, in response to moisture stress.

Established soybeans were relatively tolerant of excess moisture conditions. Soybeans grown under excess moisture consistently yielded the same as (in four of six site-years) or better than (in two of six site-years) rainfed conditions (Figure 1). This occurred even though excess moisture treatments had received substantially more water during July and August than rainfed treatments (108 to 450 mm more water at Portage, and 273 to 779 mm more water at Carberry, depending on the year) and had been irrigated to the point that chlorosis became evident in the soybean crop.

Soybeans were comparatively less tolerant of later-season moisture deficits. Withholding moisture later in the growing season (July through fall) reduced soybean

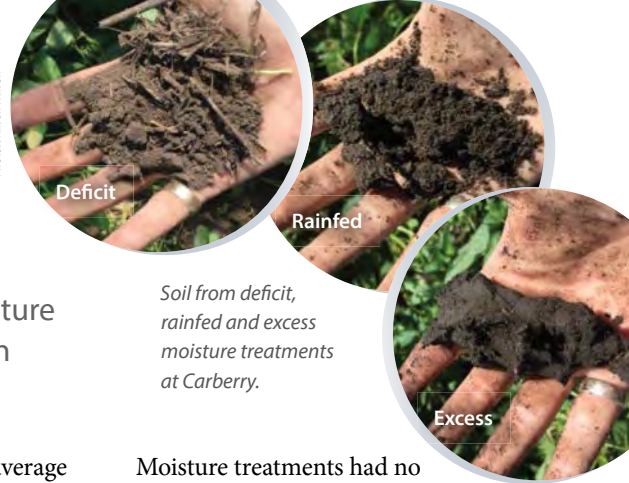
yields in four of six site-years by an average of 16–32% compared to rainfed conditions (Figure 1). Those deficit treatments received between 24–45% of the rainfall in rainfed treatments.

While there appeared to be some evidence varieties differed in their yield responses under different moisture regimes, effects were not consistent among site-years. Therefore, among the varieties tested, no varieties emerged as superior in performance across contrasting moisture conditions. Plant stress in this study, whether in the form of moisture deficit or excess, was associated with increased soybean root rot. However, while there were cases in which lower soybean yields were associated with greater root rot severity, these effects were not consistent. This suggests that factors other than root rot contributed to the yield differences observed.

Moisture treatments had no effect on seed protein in Portage in any year. However, at Carberry in all years, seed protein concentration was highest in the excess moisture treatment. Excess moisture also resulted in lower oil content in five out of six site-years.

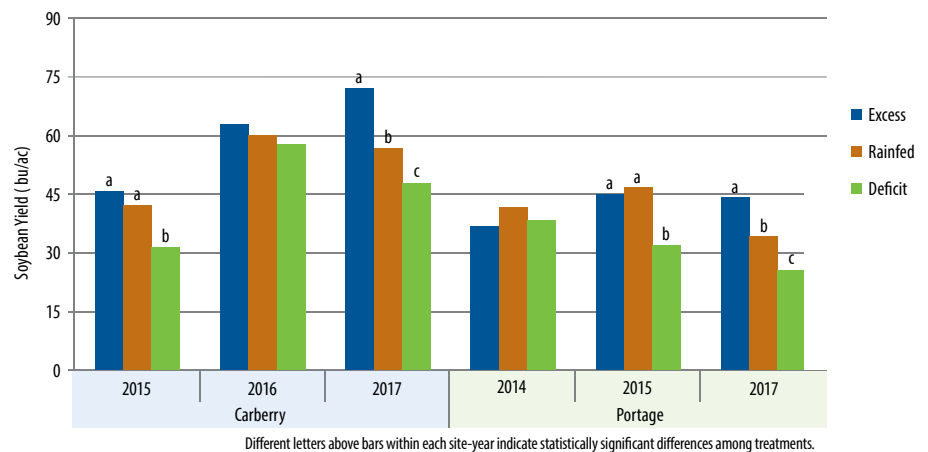
There exists the potential to explore this dataset further, to determine potential linkages between detailed soil temperature and moisture data collected over the course of the growing season, the incidence and severity of soybean root rot, and soybean yield and quality. This dataset is unique for Manitoba, as it includes a range of varieties with varying degrees of susceptibility to soybean root rot that were grown in the same field and under the same conditions, but exposed to different moisture stresses. Together, this information may contribute to a better understanding of factors driving root rot and yield under Manitoba conditions. ▀

Photos: Alison Nelson



Soil from deficit, rainfed and excess moisture treatments at Carberry.

Figure 1. The effect of three moisture regimes on soybean yield in Carberry and Portage la Prairie from 2014–2017.



PRINCIPAL INVESTIGATOR Dr. Ramona Mohr, Agriculture and Agri-Food Canada – Brandon

MPSG INVESTMENT \$94,789

CO-FUNDERS Growing Forward 2 Growing Innovation: Agri-Food Research and Development Initiative

DURATION 4 years

Protein Content Variation Among Soybeans Grown in Morden and Ottawa

Manitoba-grown soybeans had 3.4% lower protein on average than Ottawa-grown soybeans.

SOYBEAN PROTEIN IS an important seed quality component and marketing determinant. The protein basis for commodity export to China, for example, is 39% on a dry basis (% d.b.) for commodity soybeans and 48% d.b. for food-type soybeans.

However, soybean protein levels may vary among varieties and environments. Short growing seasons are expected to result in lower soybean protein content and higher oil content. Soybean seed protein and oil content values are reported by the Canadian Grain Commission from its voluntary sampling program.

However, scientific evaluation is required to assess statistical differences in seed quality between regions and soybean lines, especially early-maturing lines that have been developed more recently.

In this study, 32 soybean breeding lines were evaluated for protein and oil content at two geographically distinct sites – Morden and Ottawa – from 2015 to 2017. These 32 lines consisted of 12 early-maturing lines from Ottawa and 20 early-maturing lines from Morden.

Ottawa-grown soybeans had significantly higher protein content than Morden-grown soybeans. Average protein content of all 32 soybean lines for all three years combined, was 3.4% lower at Morden (39.8% d.b.) than at

Ottawa (43.2% d.b.) (Table 1). However, no significant differences in oil content were found between the two sites. There was some variation in protein from year-to-year at each site, likely due to differences in growing conditions. More precipitation can result in higher protein content. However, more precipitation did not always correspond with higher protein in this study, possibly due to differences in the timing of precipitation.

Morden-grown soybean lines had lower seed weight than Ottawa-grown soybeans (Table 1). Despite seed weight differences, average yields between sites were not significantly different overall (Table 1). This suggests that the inverse relationship between soybean yield and protein can

be inconsistent. The average growing season length at Morden was 117 days compared to 105 days at Ottawa. However, Morden received 91% of the corn heat units (CHU) received at Ottawa during the growing season. This may be one of many environmental factors responsible for the seed protein difference between the two sites.

Low soybean protein content is a growing concern among Manitoba farmers. The results from this study confirm that there is lower protein in Manitoba compared to Ontario likely due to environmental differences. Materials and knowledge generated by this study are useful in future soybean breeding and agronomy research. MPSG has invested in research that will address this question about seed quality differences among commercial soybean varieties and environments in which they are grown. Soybean samples from the annual, multi-location variety performance trials will be analyzed for key attributes, including crude protein, amino acid, oil and moisture content. ▶



Table 1. Soybean seed protein, oil, yield, seed weight and environmental conditions at Morden and Ottawa (2015–2017).

Site	Year	Protein (%)	Oil (%)	Yield (bu/ac)	Seed Weight (g/1000 seeds)	Precipitation (mm)	CHU
						Jun 1– Sep 30	Jun 1–Sep 30
Morden	2015	40.0	20.4	20.6	159	211	2502
	2016	41.6	20.0	41.4	174	395	2608
	2017	37.7	18.5	36.0	154	209	2695
	Average	39.8	19.6	32.7	162	272	2602
Ottawa	2015	42.2	20.2	41.7	179	359	2795
	2016	43.9	20.0	26.0	181	258	2912
	2017	43.5	19.0	35.7	200	253	2886
	Average	43.2	19.7	34.5	187	290	2864
	LSD (0.05)	0.7	0.4	4.1	9.4	–	–

PRINCIPAL INVESTIGATORS Dr. Anfu Hou, Agriculture and Agri-Food Canada – Morden

MPSG INVESTMENT \$144,000

DURATION 3 years

Improving Diagnostics and Our Understanding of Pulse and Soybean Nematode Pests

The quarantined nematode, *Ditylenchus dipsaci*, found in Canadian yellow pea shipments causing market access issues with India had been misdiagnosed. The accurately identified nematode, *Ditylenchus weischeri*, poses no threat to Canadian pulse production.



SOME NEMATODES CAN be crop pests, but pulse-nematode interactions on the prairies have not been widely studied. Nematodes are of economic importance, not only as they impact production, but also market access. India has insisted that yellow pea shipments from Canada be certified free of the parasitic nematode *Ditylenchus dipsaci*. The Canadian Food Inspection Agency (CFIA) has been conducting bulk ship monitoring and had, at a low frequency, reported the presence of the nematode, causing ships to be diverted and fumigated in southeast Asia before arrival in India. Exporters experienced delays and extra costs, resulting in lower yellow pea prices for farmers.

The team of researchers in Dr. Tenuta's soil ecology lab underwent several experiments to tackle this trade issue. They developed a molecular test to precisely identify nematodes at a species level and surveyed seed samples. From this, they found that the nematode present in yellow pea and other pulses on the prairies was, in fact, not *D. dipsaci*, but a closely related species, *D. weischeri*. The CFIA adopted their methodology, reanalyzing their past positive samples of *D. dipsaci*. Continued monitoring for the nematode has since been suspended by the CFIA, as there was no evidence of the pest after almost 15 years of monitoring yellow pea shipments.

Through greenhouse host screening and field microplot studies, the researchers also found that under normal prairie growing conditions, yellow peas, lentils, chickpeas and dry beans were not good hosts for *D. weischeri*. So why was the nematode

present in pea samples? Canada thistle was found to be a host for *D. weischeri* and infested weed seeds were present in export shipments. In further controlled environment studies, *D. weischeri* did survive and reproduce on some yellow pea cultivars at the highest temperature examined (27°C). Research supported by MPSG, APG, SPG and AAFC through the CAP Pulse Science Cluster, has been initiated to investigate how *D. weischeri* can reproduce at these higher temperatures on yellow pea. The new project also aims to mitigate trade threats by screening important crops grown in India as possible hosts for *D. weischeri*.

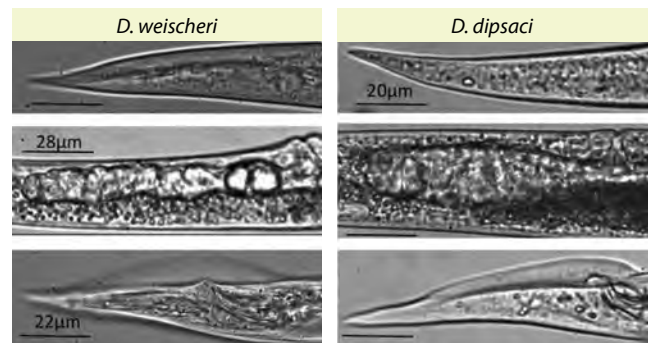
A survey of prairie pulse fields found *D. dipsaci* present in one yellow pea field in Manitoba in 2015. Garlic is susceptible to parasitism by *D. dipsaci*, and two garlic growers in Manitoba have submitted rotten bulbs to Dr. Tenuta's lab for diagnosis. Although cultivation of garlic crops is not widespread, it threatens to disperse *D. dipsaci* to yellow pea fields. Proper phytosanitation, purchase of *D. dipsaci*-free bulbs and limiting the presence of garlic

fields near pea fields are required to reduce the threat to yellow pea exports.

Researchers also found about a third of surveyed prairie fields had the nematode *Pratylenchus neglectus*. *P. neglectus* has been reported to cause yield losses in peas, lentils and chickpeas in other areas of the world, but its host range and impact on the prairies is unknown. Unlike *D. dipsaci*, *P. neglectus* does not pose a market access risk. Instead, *P. neglectus* could be causing yield losses and/or is part of the disease complex causing root rot on pulse crops. Rearing methods have been developed and on-going research will screen crop hosts and relate soil population levels to disease and yield loss in susceptible hosts.

The final activity of this cluster project developed primer sets, real-time PCR primers and protocols to quantify soybean cyst nematode (SCN) DNA in soil. This method will be an alternative to the laborious, costly and error-prone method of cyst extraction and egg counting. This diagnostic procedure also supports ongoing MPSG-funded SCN surveys led by Dr. Tenuta. ▀

Figure 1. Micrographs of *Ditylenchus* obtained from Canada thistle in Manitoba (*D. weischeri*), and garlic from Ontario (*D. dipsaci*).



PRINCIPAL INVESTIGATOR Dr. Mario Tenuta, University of Manitoba

MPSG INVESTMENT \$165,776

CO-FUNDER Agriculture and Agri-Food Canada, through Growing Forward 2 Pulse Science Cluster

DURATION 5 years

Characterizing *Fusarium* Species in Manitoba: Genetic Diversity and Detection

Molecular tools have been developed for rapid, accurate detection of *Fusarium* species. It is now possible to differentiate between three important pathogens associated with root rot in soybeans – *F. graminearum*, *F. poae* and *F. avenaceum*.

ACCURATE IDENTIFICATION OF fungal phytopathogens is essential for disease management. More specifically, rapid, accurate detection and differentiation between *Fusarium* pathogens can lead to the development of improved control options. Several different *Fusarium* species are recognized as soybean pathogens. Approximately 20 *Fusarium* species are associated with soybean root rot. However, little is known regarding the genetic diversity of these species and how to detect and differentiate them.

The objectives of this study were to:

1. examine the genetic diversity of the *Fusarium* community associated with soybean root rots in Manitoba and
2. design molecular markers to detect and differentiate these pathogens.

Plant diseases have traditionally been identified by culture-based morphological approaches that are time-consuming, laborious and require extensive knowledge of taxonomy. Using this method, diseases may be difficult to culture, identify and quantify. Molecular methods, on the other hand, offer faster, more specific,

more sensitive and more accurate results. In this study, 11 different species of *Fusarium* were identified by molecular techniques and assessed for diversity. The information gathered across *Fusarium* species was sequenced and made publicly available to other researchers for future studies.

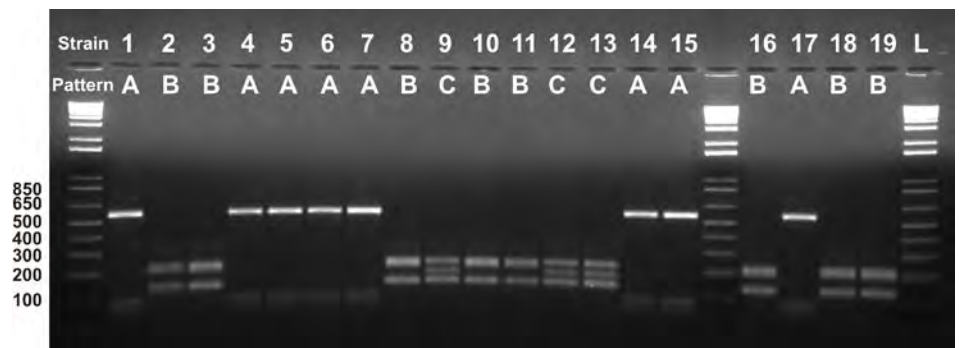
Several molecular tools were developed during this project for rapid detection of *Fusarium* pathogens that cause root rot in soybeans. One highlight is the development of a polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) protocol, which is considered one of the most sensitive methods for accurate species characterization. With the PCR-RFLP protocol developed, it is now possible to differentiate between three important soybean *Fusarium* pathogens: *F. graminearum*, *F. poae* and *F. avenaceum*.



Another highlight of the study is that the first molecular marker for specific detection of *F. graminearum* was developed. This genetic marker allows for differentiation among closely-related pathogens in the *F. graminearum* species complex and among other *Fusarium* species that can cause head blight in cereals and root rot in soybeans.

The results of this study can be used in future basic and applied research to accurately identify, understand and manage various *Fusarium* species. Further investigation is needed for *Fusarium* species associated with soybean seed. Due to the cross-pathogenicity across crop types and widespread production of legume and cereal crops, *Fusarium* management will become increasingly important. ▀

Figure 1. Differentiation between *Fusarium* species according to the PCR restriction fragment length polymorphism (PCR-RFLP) method. Pattern A is *F. avenaceum*, pattern B is *F. graminearum* and *F. cerealis*, and pattern C is *F. poae*.



PRINCIPAL INVESTIGATOR Dr. Fouad Daayf, University of Manitoba

MPSG INVESTMENT \$47,200 – two objectives

CO-FUNDERS Manitoba Wheat and Barley Growers Association, Mitacs

DURATION 2 years

Characterizing Fusarium Species in Manitoba: Cross-Pathogenicity, Competitiveness and Mycotoxins

Fusarium strains that cause *Fusarium* head blight in wheat, barley and oats can also cause root rot in soybeans. Species from oats and soybeans were the most aggressive.

SOYBEAN CROPS ARE susceptible to diseases caused by different *Fusarium* species in the forms of root rot and wilting. *Fusarium graminearum*, the specific species of *Fusarium* that causes head blight in cereals has been reported to also infect soybeans and peas. Therefore, it was important to investigate if infection of cereal crops by common species of *Fusarium* is contributing to root rot infection in soybeans and peas, or vice versa. Cross-pathogenicity of *Fusarium*, or the ability of this disease to infect different crop types, would indicate a greater risk of disease development over the long term and reduce the usefulness of crop rotation as a management tool.

The objectives of this project were to:

1. study the cross-pathogenicity of *Fusarium* species between soybean and cereal crops,
2. investigate the competitiveness of *Fusarium* species isolates from soybeans/peas and cereals and
3. investigate the specific toxin-producing potential of *F. graminearum* isolates from soybeans versus cereals.

Fusarium isolates belonging to 11 different species were found to commonly infect cereals, soybeans and peas confirming cross-pathogenicity. Moreover, head blight-causing *Fusarium* strains were shown to induce root rot symptoms in soybeans. Disease symptoms were present in soybeans at V3 (3rd trifoliolate) and ranged in severity from 0.5 to 8.3 on a scale of 0–9. Results showed that *F. graminearum* from oats and *F. avenaceum* from soybeans were the most aggressive species. This study was

also the first ever to report *F. cerealis* as a cause of root rot in soybeans.

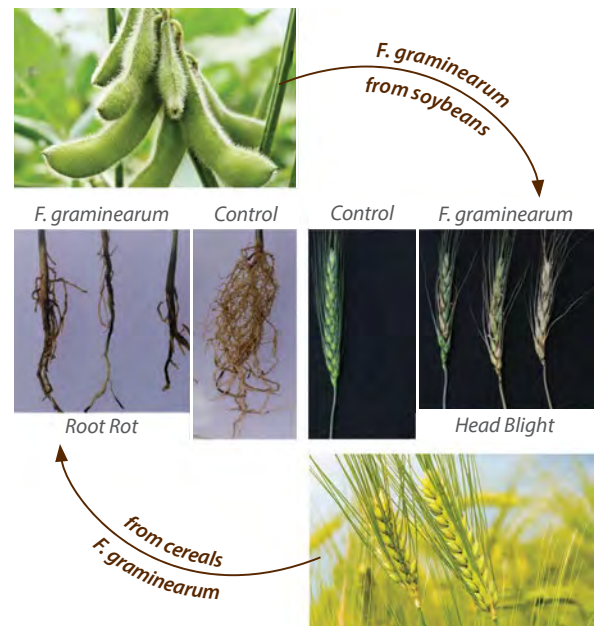
The competitiveness of *F. graminearum* isolates was assessed both in petri dishes and on plants. Petri dish tests showed that *Fusarium* strains isolated from wheat inhibited the growth of strains from other crop types. On soybean plants, the soybean-derived *Fusarium* isolate caused the most severe root rot compared to the wheat-derived isolate.

Several *Fusarium* species in addition to *F. graminearum* are known to cause root rot in soybeans, including *F. avenaceum*, *F. poae*, *F. cerealis*, *F. culmorum*, *F. sporotrichioides*, *F. acuminatum*, *F. redolens*, *F. incarnatum* and *F. equiseti*. Most of these species are also known to produce mycotoxins (e.g., trichothecenes such as DON), which can impact both human and livestock consumers. Due to this, the

mycotoxin production potential of select *Fusarium* species was assessed. Some *F. graminearum* isolates from soybeans in this study showed potential to produce toxins. However, further investigation is required to assess other species and understand their impact.

Among the root rot pathogens that infect soybeans, *Fusarium* is the most common in Manitoba. However, infection levels are still low in the province due to the relatively short history of soybeans. Knowledge of cross-pathogenicity from this study is especially useful for crop rotation planning, as soybeans are commonly grown in rotation with cereals in Manitoba. Longer rotations with greater crop diversity may reduce infection and delay the spread of pests. However, longer rotations require more diverse marketing and management strategies. ▀

Figure 1. Cross-pathogenicity of some *Fusarium* species between wheat and soybeans. *F. graminearum* isolated from soybean plants caused head blight in wheat and *F. graminearum* from barley caused soybean root rot.



PRINCIPAL INVESTIGATOR Dr. Fouad Daayf, University of Manitoba

MPSG INVESTMENT \$47,200 – two objectives

CO-FUNDERS Manitoba Wheat and Barley Growers Association, Mitacs

DURATION 2 years

Defining and Refining the Critical Period of Weed Control for Soybeans in Manitoba

The critical period to control weeds to avoid yield loss in soybeans was shortened significantly by planting regionally competitive varieties at narrow row widths and higher plant populations. The weed-free period ended on average between V2 to V4, but ranged from VE to R1.

THE CRITICAL PERIOD of weed control (CPWC) is the period of time a crop must remain free from weeds to prevent yield loss. Defining this period for soybeans in Manitoba allows farmers to ensure that only in-crop herbicide applications that are necessary are applied, making soybean production more profitable.

OBJECTIVE 1: DEFINE THE CPWC

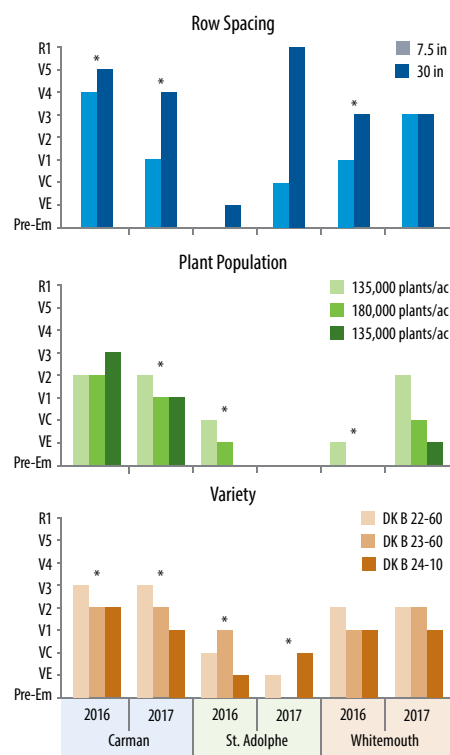
First, to determine the critical time of weed removal at the beginning of the CPWC, soybeans were kept weedy for specific lengths of time. Once weed control began, plots were kept weed-free. This determined when the weeds emerging with the crop began to cause yield loss and needed to be controlled.

In a second experiment, the critical weed-free period defines the end of the CPWC. Here, plots were kept weed-free for increasing periods of time after planting to determine at which stage weeds no longer caused yield loss. Beyond this point, an in-crop herbicide was no longer necessary.

Determining the start of the CPWC proved challenging in farm fields. Due to low, inconsistent weed pressure, resulting low yield losses made it difficult to mathematically establish a clear beginning.

The average end of the CPWC was between V2 and V4. Overall, the end of the CPWC ranged between VE and R1 (Figure 1). This means at some sites, under low weed pressure, an in-crop herbicide was not necessary, while at others under high weed pressure, the crop needed to remain free from weeds until R1. Different soil characteristics, environmental conditions and weed populations help explain the wide range in the end of the CPWC.

Figure 1. End of the critical weed-free period for soybeans in Manitoba for three experiments with an acceptable yield loss threshold of 5%. Unless stated otherwise, DKB 23-60 was grown at 180,000 plants/ac on 15" rows.



*Indicates site-years where the treatment effect was significant at $p < 0.05$.

OBJECTIVE 2: SHORTEN THE CPWC

This experiment determined if elements of an integrated weed management approach could improve soybean competitive ability and shorten the length of time soybeans must remain weed-free. Specific management tools evaluated included variety, row spacing and plant population.

In general, the CPWC ended one to three stages earlier in narrow-row than wide-row soybean production (Figure 1). Planting soybeans in narrow rows created a more competitive crop that consistently shortened the CPWC, especially under high weed pressure.

Adjusting plant populations was another cultural management practice that reduced the length of time soybeans must be kept weed-free. This tool was most effective under low to moderate weed pressure. Reducing plant populations to 135,000 plants/ac lengthened the CPWC by one growth stage. Increasing plant populations to 270,000 plants/ac did not shorten the end of the CPWC, but this provided yield stability when weed pressure was high.

The influence of soybean variety on the end of the CPWC was site-specific, but consistent across years. This indicated that the ability of soybean varieties to compete with and perform under weed pressure was region-specific.

Integrating these weed management tools as standard soybean production practices will reduce the need for multiple in-crop herbicide applications, lowering production costs. This greatly reduces the risk for developing herbicide-resistant weeds, especially glyphosate-resistant weeds that have become prominent in more established soybean growing areas.

Further research is needed to refine the CPWC across Manitoba for a broader range of weed species and densities. Other weed management strategies, such as planting dates and soil fertility, could further influence the competitive ability of soybeans and should be considered. ▀

Residue Management Following Soybeans

With appropriate seeding equipment, it is possible to eliminate or reduce tillage after soybean harvest without negatively affecting spring seedbed conditions or following crop yields.

FARMERS THROUGHOUT MANITOBA are investing resources and time incorporating soybean residues in the fall using varying amounts of tillage. In other soybean growing regions in North America, farmers most often direct seed subsequent crops into soybean stubble. The purpose of this project was to evaluate residue management options (i.e., tillage) following soybeans in Manitoba.

On-farm experiments were established in the fall after soybean harvest, from 2013 to 2017 in five fields near Boissevain, Winkler, Carman, Landmark and New Bothwell.

Four tillage treatments were compared from 2013 to 2015:

1. deep-till cultivator or double disc tillage,
2. no tillage or direct seeding,
3. vertical tillage – low disturbance (discs set on 0° angle so that residue is somewhat incorporated but mostly left on the soil surface) and
4. vertical tillage – high disturbance (discs set on a 6° angle so that residue is incorporated with little residue left on the soil surface).

The impact of these tillage treatments on spring seedbed conditions (temperature, moisture) and on the plant stand and yield of subsequent wheat, corn and soybean crops were evaluated. In the last year of

the experiment (2016), this approach was simplified to become a part of the MPSG On-Farm Network. This meant each farmer's standard tillage method for soybean residue was compared to direct seeding into soybean stubble.

There were remarkably few differences between soybean residue management treatments in this four-year study. Once the next crop was planted, it was often hard to distinguish treatments within the field (Figure 1).

Following soybean harvest in the fall, soybean residue provided 40–88% ground cover in the no-till treatments. This ground cover decreased 31–57% by the following spring. This means soybean residue can be expected to breakdown between harvest and spring planting, even when residue is left unincorporated on the soil surface.

In the spring, soil moisture and temperature at a seeding depth of 5 cm were recorded for each treatment over the emergence period of the following crop. No significant differences in soil moisture nor temperature were found between residue management treatments at any site.

There were no differences in test crop stand nor test crop yields between soybean residue management treatments in four out of five experiments. Dry conditions following corn planting near Carman in 2016 resulted in uneven corn emergence

and differences in final plant stands among treatments.

For the experiment at Landmark in 2017, the subsequent soybean test crop yield was three bushels per acre higher in the fall tillage treatment than in the direct seeding treatment. However, there were no differences in plant stand, soil temperature or moisture to explain this yield difference.

With appropriate seeding equipment, it is possible to eliminate or reduce tillage after soybean harvest. This finding is especially important given the wind erosion events that have occurred frequently across southern Manitoba over the winter and in early spring.

Decisions about residue management are always farm, field and equipment-specific, but the results of this on-farm study suggest that conventional tillage of low-residue crops such as soybeans may not be necessary in Manitoba, regardless of soil type.

Some of the concerns about direct seeding into soybean residue that were not addressed in this project should be investigated further, such as the impact of ruts after harvest and seeding equipment or openers for planting directly into soybean residue. The financial and time costs of residue management, as well as the risk of soil loss from erosion after soybeans, are good reasons to test your own residue management ideas on your farm. ▶

Figure 1. Soybean residue management treatments near Winkler in 2015.



Vertical Till – High Disturbance

Vertical Till – Low Disturbance

Conventional Till – Deep Till Cultivator

No Till – Direct Seeding

PRINCIPAL INVESTIGATOR Dr. Yvonne Lawley, University of Manitoba

MPSG INVESTMENT \$70,285

CO-FUNDER Western Grains Research Foundation, *Growing Forward 2*
Growing Innovation: Agri-Food Research and Development Initiative

DURATION 4 years

The Secret to Cooking Whole Beans: A Scientific/Culinary Investigation

Soaking dry beans in a salt brine, or adding 1% salt while cooking beans, reduced cook time by 12–14% while improving flavour and texture.

CONFLICTING INFORMATION IS extensive in bean cookery. Cookbooks, industry guidelines, chefs and even packaging directions provide different options for adding salt when cooking beans.

Traditionally, the belief has been you should not add salt to beans until after they are cooked or else they'll have an unpleasant, grainy texture. Many culinary experts still opt to use salt, but disagree on when it should be added or in what amount. This project set out to create better guidelines for cooking dried pulses.

This study reviewed average cook times along with changes in flavour and texture with varying amounts of salt introduced in contrasting methods. The effects of salt addition while cooking, salt brine soaking and water quality (i.e., water hardness) on the final qualities of a cooked bean were evaluated for navy, black, pinto, kidney and faba beans.

Properly cooked beans have a slightly firm bite and a smooth, creamy texture that is not watery or gritty. They should appear shiny with bright colour and not be broken when cooked. The flavour should be earthy and not salty.

First, average cook times were determined using a Mattson cooker apparatus, which is a quantitative method

that measures the time it takes to puncture 80% of the beans with weighted plungers. This was then compared to an in-kitchen chef evaluation of doneness. Finally, consumers tested cooked beans with two salt treatments – salted brine and salt in the cook water against an unsalted control, to determine if salt negatively affected sensory characteristics when added during cooking.

Salt played an important role in the finished texture and flavour development of beans. Brined and 1% salt addition treatments improved the flavour and texture of beans, while reducing the cooking time by 12–14%. Texture improved once beans cooked with salt were drained and cooled for 2–4 minutes. More specifically, black beans in brined treatments had better colour retention with smoother seed coats, enhancing appearance. In consumer taste trials, the unsalted control beans were the least favourite texture, dispelling the notion that salt in the cook water creates an undesirable texture.

On the other hand, too much salt had a negative impact on cooking beans. An increase of salt in the 2–3% range, toughened the seed coat and resulted in an unpleasant, gritty texture. This was most apparent with kidney and faba beans where 2–3% salt additions increased average cook time and reduced the acceptability of the cooked bean.

Water chemistry (both soft and hard water) played a significant role in increasing cook time and decreasing texture of finished cooked beans when compared with distilled water. Cooking

DEVELOPED FROM THIS STUDY

Cooking Recommendations for Beans

2% BRINE SOAK

Prepare by adding 2.5 teaspoons (15 g) of salt to 3 cups of water, stir until fully dissolved.

Navy Beans

average cook time = 34 minutes

Black Beans

average cook time = 28 minutes



1% SALT IN COOKING WATER

Prepare by adding 1.5–2 teaspoons (10 g) to 4 cups of fresh, distilled boiling water.

Faba Beans

average cook time = 9 minutes

Kidney Beans

average cook time = 36 minutes

Pinto Beans

average cook time = 26 minutes

beans with hard water resulted in longer cook times than cooking with soft water.

Although chefs prefer cooking pulses using a pressure cooker, home preparation by boiling is still the most accessible method. The results from this project may be incorporated into existing recipes to provide better pulse experiences by adding some salt while cooking beans to balance the flavour of the final dish. Using these results, Red River College will produce a set of guidelines to cook pulses, engaging students in the learning process to ensure the next generation of chefs understand how to make the perfect pulse plate. ▶

PRINCIPAL INVESTIGATOR Joel Lamoureux, Red River College

MPSG INVESTMENT \$8,209

CO-FUNDER Natural Sciences and Engineering Research Council (NSERC) – Red River College

DURATION 1 year

Exploring Market Diversification: Value-Added Fermentations of Peas

Manitoban peas, in the form of pea meal, pea flour or purified pea protein may be used as a nitrogen source for industrial lactic acid, ethanol and antibiotic fermentations.



Pea flour and purified pea protein are being investigated as an inexpensive nitrogen source in three commercial fermentations.

ENERGY, FUEL, PLASTICS, chemicals and medicines have the potential to be derived from plant biomass rather than fossil fuels. Already, starch sourced from corn and wheat are used to produce ethanol for fuels while soybeans, canola and corn provide oil for biodiesel production.

Industrial fermentations produce fuel ethanol, pharmaceuticals and lactic acid. The latter is used as a green solvent, food acidulant and as a raw material for biodegradable plastic. New fermentation processes are being developed to produce solvents, fuels, plastics and chemical industry feedstocks. For these fermentations to occur, a source of nitrogen (N) is required. Given their high protein content, peas should be an excellent source of N for fermentation microorganisms.

This project investigated the use of pea flour and purified pea protein as an inexpensive N source in three typical commercial fermentations, including antibiotic production, lactic acid production (polylactide plastics) and as a fermentation-promoting ingredient in fuel and beverage ethanol fermentations. These fermentations are either high-volume or produce high-value products and would provide a valuable new market for Manitoba pulses.

LACTIC ACID

Pea flour and purified pea protein supported the growth of lactic acid bacteria (LAB). Rates and concentrations of lactic acid produced by these bacteria were comparable to the expensive conventional media. In addition, LAB are used in the food processing industry as

inoculants in cheese, yogurt, sauerkraut, pickles and ready to eat meats, such as salami. Pea protein could provide a competitive source of nitrogen for these relatively high-value fermentation products. This fermentation would also seem to be the most likely opportunity to incorporate peas as a media ingredient.

ETHANOL

Growth and production of ethanol by *Saccharomyces cerevisiae* (brewer's yeast) was successful using pea flour and purified pea protein. The use of this high-protein fermentation media resulted in increased rates of fermentation and increased the absolute concentration of ethanol.

For lower-value products, such as fuel ethanol, where the raw material (typically wheat or corn) provides sufficient nitrogenous nutrients, it is unlikely to be cost effective to add pea protein. However, the ability of pea protein to overcome yeast inhibition at high substrate concentrations may make it economical for fuel ethanol producers to add pea protein in return for shorter fermentation run times and higher productivity over the long term.

ANTIBIOTIC

Pea flour and purified pea protein successfully supported the growth of antibiotic-producing organisms, but had mixed results in the production of antibiotics. In this study, pea protein increased antibiotic production in one case, but failed to in another. A wider array of antibiotic-producing microorganisms will need to be tested to understand the role of peas as fermentation media for antibiotic production.

BIOTECHNOLOGY APPLICATIONS

The production of recombinant proteins for human therapeutic use is already a multi-billion-dollar industry and the most rapidly expanding field in fermentations, located mainly in North America and Europe. The organisms used to produce recombinant proteins are *Pichia pastoris*, *Escherichia coli*, *S. cerevisiae* and Chinese hamster ovary (CHO) cells. As part of this experiment, *P. pastoris* and *E. coli* were examined. Pea protein successfully supported their growth. Many other bacteria and fungi could also be grown using either pea protein or hydrolyzed pea protein as their nitrogen source.

These results show that Manitoba peas, and possibly other pulses and their purified proteins, could find a market as a fermentation ingredient providing the nitrogen requirement for many microorganisms. Further research needs to be done to determine whether this would be economically viable. ▀

PRINCIPAL INVESTIGATOR Dr. Paul Holloway, University of Winnipeg

CO-FUNDER Agri-Food Research and Development Initiative

MPSG INVESTMENT \$32,860

DURATION 3 years

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Photo by Laura Schmidt, MPSC

