GRAIN RESEARCH LABORATORY
Annual Program Report
2018

Canadian Grain Commission   Commission canadienne des grains

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Canadian Grain Commission
MANDATE

The Canadian Grain Commission works in the interests of grain producers. Guided by the Canada Grain Act, the Canadian Grain Commission works to establish and maintain standards of quality for Canadian grain, regulate grain handling in Canada, and to ensure that grain is a dependable commodity for domestic and export markets.

Grain Research Laboratory
VISION

Advancing science for better nutrition and lives through high-value, quality grain.

Grain Research Laboratory
MISSION

- Be the pre-eminent provider of science to support Canada's grain quality assurance system

- Enhance the marketability of Canadian grains through scientific research, monitoring and analytical services

- Anticipate and respond to the needs of the grain value chain, through interaction with the grain sector

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Director's welcome

Dr. Stefan Wagener
Director, Grain Research Laboratory

"Today's scientific analyses, research and technology projects by the Grain Research Laboratory are tomorrow's grain quality and safety assurance for Canada."
– Stefan Wagener

2018 was another exciting year for the Grain Research Laboratory, and it is my pleasure to present to you our Annual Program Report. This edition highlights some of the unique areas, projects and developments within the Canadian Grain Commission's Grain Research Laboratory and participating scientific programs.

One of our most notable projects in 2018 was our combined response with the Canadian Food Inspection Agency and other government organizations in the detection and monitoring of an isolated genetically modified wheat event in Alberta. Thanks to years of variety identification monitoring by the team of Dr. Daniel Perry and the detection work by Dr. Tigst Demeke and his staff, our trading partners and stakeholders were reassured that there is no genetically modified wheat in Canada's commercial handling system.

Another highlight of 2018 was the enhancement of our Harvest Sample Program. In addition to quality results traditionally provided on harvest samples, producers who participated in the 2018 Harvest Sample Program also received test results for falling number and deoxynivalenol (DON) on their wheat and durum samples. As in 2017, the Grain Research Laboratory and the Canadian International

Grains Institute combined efforts in assessing the 2018 wheat and durum harvest quality. This very successful collaboration allowed for timely results that were presented during the 2018 New Crop Missions in partnership with Cereals Canada.

As we look forward to 2019, we also recognize the changes that are occurring within the Grain Research Laboratory. Dr. Tom Gräfenhan will be leaving the Canadian Grain Commission to pursue a new career opportunity at the Public Health Agency of Canada's National Microbiology Laboratory. We wish him all the best and thank him for his great service and contribution.

I encourage you to read the report and learn more about the abilities, research areas and the quality of services our dedicated scientists and staff at the Grain Research Laboratory provide on a daily basis. Please share with us your comments and thoughts. We greatly appreciate your feedback.

Grain Research Laboratory

The research conducted by the Canadian Grain Commission's Grain Research Laboratory falls under two categories: crop research and technology research.

Research related to crops allows us to assess Canadian grain harvest quality and studies how grading factors affect end-use properties. Crop research also develops new uses for Canadian grain and evaluates new varieties as part of the variety registration process.

Research related to technology evaluates and develops methods used to assess the quality and safety of Canadian grain.

Crop research programs include:

- Bread and Durum Wheat Research
- Milling and Malting/Research on Barley and Other Grains
- Oilseeds
- Pulse Research
- Wheat Enzymes, Asian Products and Analytical Services

Technology research programs include:

- Grain Biotechnology Research
- Microbiology
- Trace Organics and Trace Elements Analysis
- Variety Identification Research and Monitoring

Beyond each program's own testing and research, all of the programs support four key activities:

Cargo quality monitoring

Provides analytical testing of export grain shipments (e.g. mycotoxins, pesticides, variety composition) to ensure they meet Canada's grading and quality parameters.

Harvest Sample Program

Producers send in a voluntary sample of their harvest, and in return receive a personalized report on the quality of their crop.

Requests for service analysis

Provides analytical services of samples submitted by the industry for testing, at times for a fee.

Plant breeder line evaluation

Provides testing and recommendations for the advancement of breeder line seed.

Statistics and facts
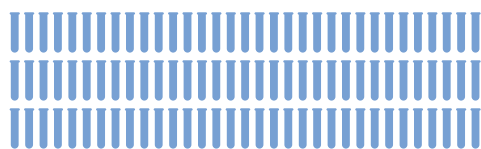
Harvest Sample Program

We publish annual harvest and crop reports. We also publish an annual Fusarium survey report from samples we collect through the Harvest Sample Program.

The Harvest Sample Program received

15,135 samples for the 2018–19 crop year

We conducted **8,870** falling number and DON tests on wheat

We tested **1,483** cargo shipment samples

We conducted **511** tests for **service requests by external clients**, which included milling of **80** samples

The **most popular requests** were for **Nitrogen/Protein** by combustion and **Fatty acid Profile**

Currently, we use **117** different **test methods**

6 scientific articles and **1 book chapter** were published by our scientists

22 scientific presentations were delivered by our scientists

Out of **20 grains** regulated by the Canadian Grain Commission, we analyze **14 different types** of grain:

- Peas
- Lentils
- Wheat
- Durum
- Barley
- Oats
- Buckwheat
- Chickpeas
- Beans
- Mustard
- Flaxseed
- Rye
- Soybeans
- Canola/rapeseed

Advancing Crop Science

At the Canadian Grain Commission's Grain Research Laboratory, we're constantly developing new ways to help improve the quality of grains and of the products they are used to make. Through crop science and technology research, we're doing our part to support innovation in Canada's grain sector, from farm to global consumer.

Bread and Durum Wheat Research

Dr. Bin Xiao Fu

Supporting Canadian wheat quality improvement:
A rapid protocol for assessing key quality traits

Efficiently and effectively selecting key quality traits is crucial for developing new wheat varieties with improved end-use quality. However, the traditional test protocol is time-consuming and requires large samples. To address these issues, we have developed a new protocol that generates fast, accurate results with the use of smaller samples.

Factors in wheat quality

Protein content and gluten strength are key to wheat quality, controlling flour water absorption, mixing requirements, and dough viscoelasticity. The viscoelasticity, or balance between extensibility and elasticity, in dough determines how the wheat can be used and the quality of the end product. High-protein wheat tends to have strong gluten, suitable for bread. Low-protein wheat tends to have weak gluten, suitable for pastries.

Milling quality affects wheat's commercial value, and is complicated to assess. Physical properties such as hardness, kernel weight, test weight, soundness, and vitreousness affect how readily wheat grain mills into flour. The key indicators of milling quality are yield, ash content, and bran contamination. Milling yield is predicted by milling a sample.

The farinograph measures absorption, mixing time, and dough stability during mixing. The extensograph assesses dough handling, fermentation, and baking properties.

1

Traditional protocol

The traditional protocol for evaluating milling performance and dough properties uses a Bühler test mill, a farinograph, and an extensograph (images 1 to 3). This method is time-consuming for the milling and baking industry and, because it requires 2 to 5 kg of wheat, it can't be used to screen breeding populations, which involves testing many very small samples.

2

New protocol

Predicting flour yield using a modified Quadrumat Junior (QJ) milling protocol

We developed a modified QJ milling protocol to predict flour yield using just 200 g of wheat. The results strongly correlated (r > 0.89, p < 0.001) with the Bühler test mill for selected advanced breeding lines grown in 2015, 2016, and 2017 (Figure 1).
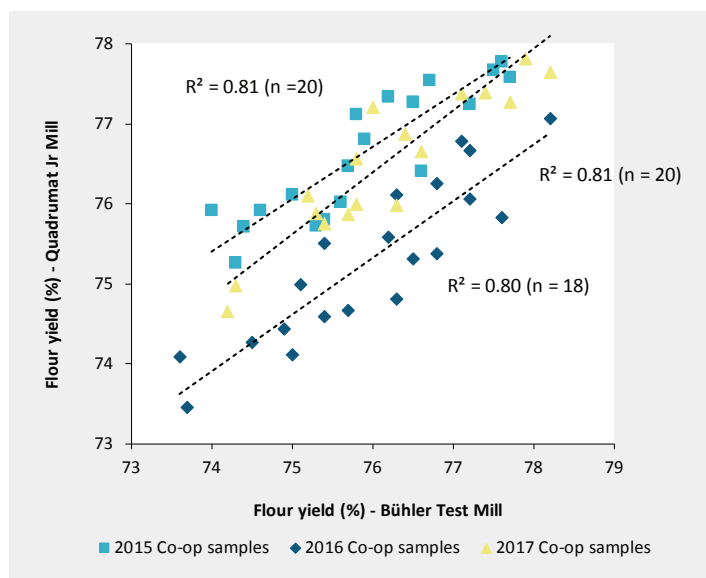
Figure 1 Relationship of flour yield between Bühler test mill and proposed Quadrumat Junior milling protocol for selected advanced breeding lines.

Predicting flour water absorption with GlutoPeak

Using the GlutoPeak, we used 8 g of QJ milled flour to predict water absorption as measured by farinograph. Samples from 32 advanced breeding lines with a range of flour water absorption were tested. GlutoPeak maximum torque of QJ milled flour strongly correlated (r = 0.91, p < 0.001) with farinograph water absorption of Bühler milled flour (Figure 2).
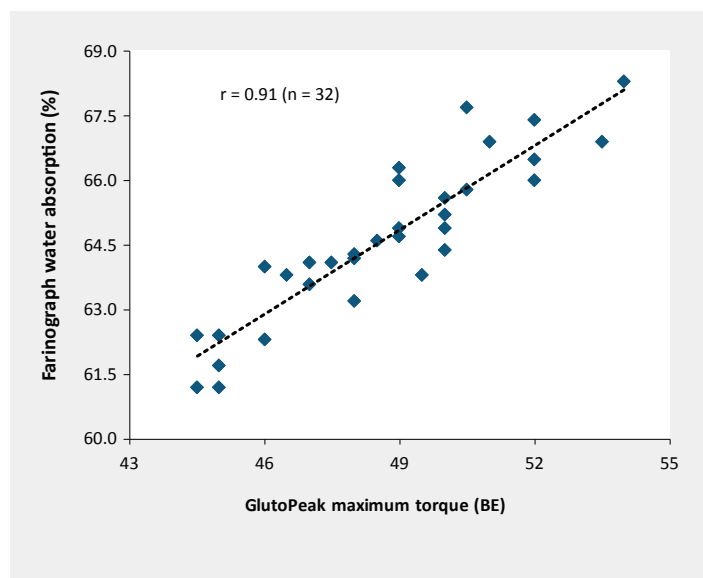
Figure 2 Relationship between farinograph water absorption and GlutoPeak maximum torque for selected advanced breeding lines.

Images
[1] Bühler test mill
[2] Farinograph
[3] Extensograph

Determining gluten strength with GlutoPeak and rapid extensograph

Assessed using just 8 g of flour, GlutoPeak peak area and strength index results strongly correlated with dough strength parameters as measured by farinograph, mixograph, and extensograph (Table 1). We conducted rapid extensograph (up to three times faster than standard extensograph) at two water absorption (WA) levels: constant WA of 67.5%, and GlutoPeak-predicted WA + 2%. Rapid extensograph mixing time, mixing energy, extensograph Rmax, and area results strongly correlated with the modified extensograph method. (Table 2).

Table 1   Simple correlation coefficients (r) between GlutoPeak peak area (PA) and strength index (GSI) and major flour quality parameters for selected advanced breeding lines

GlutoPeak Parameters		Max Torque	PA	GSI
Farinograph Parameters	Absorption	**0.91*****	-0.37*	-0.15 NS
	Development time	-0.10 NS	0.72***	0.73***
	Stability	-0.02 NS	**0.76*****	**0.79*****
Mixing and Extensograph Parameters	Mixing time	-0.16 NS	**0.89*****	**0.89*****
	Mixing energy	-0.04 NS	**0.76*****	**0.78*****
	Max. resistance	-0.12 NS	**0.89*****	**0.91*****
	Extensibility	0.20 NS	-0.55**	-0.52**
	Area	-0.06 NS	**0.85*****	**0.87*****

*, **, *** = significance at 5, 1 and 0.1% levels, respectively; NS = not significant (*P* > 0.05)

Table 2   Simple correlation coefficients (*r*) for dough properties measured by rapid extensograph method at constant and adjusted water absorption as compared to modified extensograph method
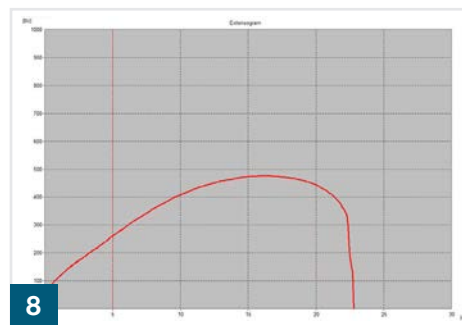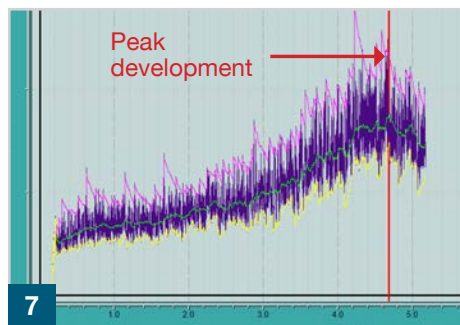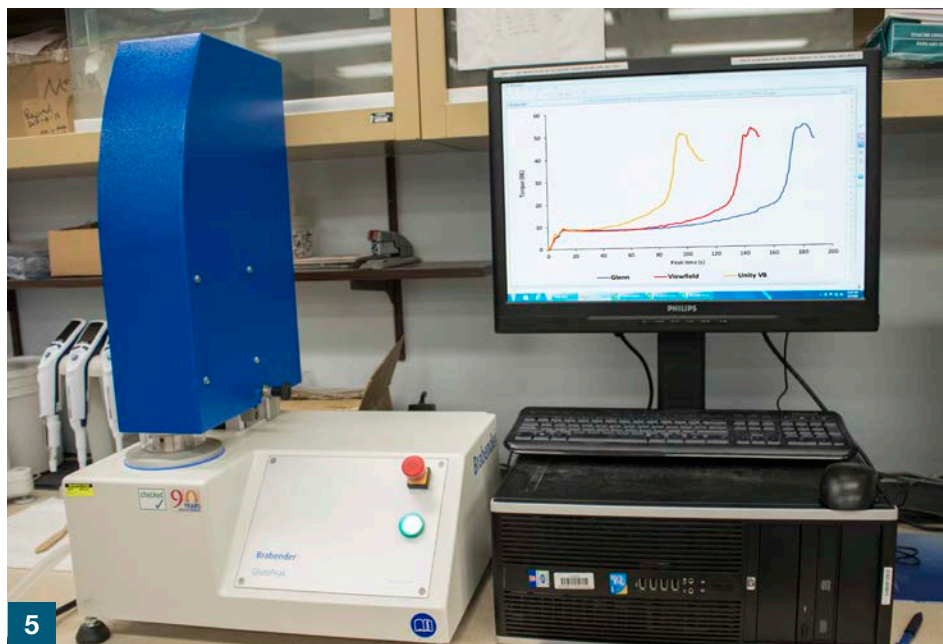
Rapid extensograph		Modified extensograph				
		Mixing time	Mixing energy	*Rmax*	Extensibility	Area
Constant WA	Mixing time	**0.89*****	0.87***	0.93***	-0.62**	0.86***
	Mixing energy	0.83***	**0.88*****	**0.88*****	-0.54**	0.82***
	Rmax	0.90***	0.92***	**0.93*****	-0.54**	0.89***
	Extensibility	-0.27 NS	-0.36 NS	-0.25 NS	**0.51***	-0.12 NS
	Area	0.88***	0.87***	0.93***	-0.45**	**0.92*****
Predicted WA + 2%	Mixing time	**0.92*****	0.85***	0.91***	-0.58**	0.85***
	Mixing energy	0.83***	**0.80*****	**0.83*****	-0.46**	0.80***
	Rmax	0.94***	0.92***	**0.97*****	-0.58**	0.92***
	Extensibility	-0.19 NS	-0.16 NS	-0.16 NS	**0.37 NS**	-0.07 NS
	Area	0.92***	0.90***	0.95***	-0.52**	**0.92*****

*, **, *** = significance at 5, 1 and 0.1% levels, respectively; NS = not significant (*P* > 0.05)

Meaningful results for wheat breeders

The new protocol we've developed is up to four times faster and uses just 200 grams of wheat to predict milling performance, flour water absorption and gluten properties (images 4 to 8).

This protocol can be widely adopted to screen key quality traits in wheat breeding programs. In order to align quality selection with the requirements for registration, we've proposed this new protocol to Canadian wheat breeders for screening wheat flour yield, flour water absorption and dough properties in their breeding programs.

Peak development

Images
[4] Quadrumat Junior (QJ) mill
[5] GlutoPeak
[6] Rapid Extensograph
[7] Dough Mixing Curve
[8] Rapid Extensograph Curve

Wheat Enzymes, Asian Products and Analytical Services

Dr. Dave Hatcher

Assessing the effect of Fusarium on end-use functionality

We've developed a procedure that may allow inspectors to assess Fusarium damaged kernels (FDK) so that both grain safety and end-use functionality concerns caused by Fusarium damage are addressed.

Background

Currently, the grading tolerances for FDK are based on grain safety concerns, rather than on the effect of Fusarium on end-use functionality. This is because Fusarium *graminearum* produces the mycotoxin deoxynivalenol (DON), which is toxic to humans and animals. However, other species of Fusarium, such as *Avenecium*, do not produce DON.

All species of Fusarium produce enzyme proteases, which degrade the quality of products made from affected wheat. Proteases are enzymes that attack glutenin and gliadin proteins which, when mixed with water to make dough, form the critical polymer gluten. In bread dough, gluten retains the carbon dioxide produced by yeast, allowing the bread to rise and keep its shape during baking. Changes to the amount and composition of gluten will affect the dough's strength and extensibility.

Effect of Fusarium-damaged kernels on flour quality

At this time, FDK is assessed visually. Inspectors look for kernels that show signs of Fusarium damage such as white, chalky kernels that are distorted in shape. We've found that these kernels also have excessive fungal protease activity.

Currently, the allowable percentage of FDK in Canada Western Red Spring wheat is 0.25% in No. 1, 0.80% in No. 2, 1.5% in No. 3 and a maximum of 4.0% for Feed. Preliminary studies on flour made with different amounts of distorted, chalky kernels in wheat are seen in Figure 1. The impact on flour strength (RMax) is clearly evident at 0.8% FDK and increases as FDK percentage increases in the wheat grist.

Method

To measure DON levels in a sample of wheat, we first mill the sample using a falling number mill to produce whole meal. We then extract a sample with water. Finally, we use a Raptor ® Lateral Flow Strip method to measure DON levels.

Currently, we're investigating if we can combine DON testing with end-use functionality testing by using the same water extract for both tests. We're using a new assay with specialized compounds to assess FDK protease levels in the prepared sample. Our initial studies using standard chromophoric protease substrates (target of the protease) did not yield the sensitivity required to detect Fusarium proteases in flour milled from FDK damaged wheat (Figure 2). Subsequent research with a specifically modified substrate (protein) which fluoresces when cleaved by the protease indicated a linear relationship between fluorescence and the wheat flour's FDK %.

While the substrate indicates a "proof of concept" and excellent reproducibility, it doesn't produce results quickly enough to be useful for grading. We are now researching making synthetic fluorescent proteins to find out if these will shorten the assay time and increase sensitivity. Knowing the protease levels allow us to determine their effect on the end-use functionality of Fusarium-damaged kernels.

We are also using a mass spectrometer to identify the key amino acid sequences where these proteases attack the gluten protein (Figure 3) to help design our synthetic proteins substrates.
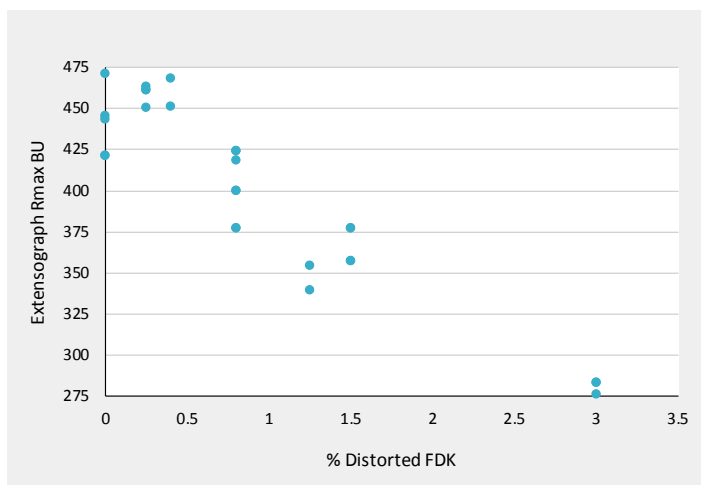
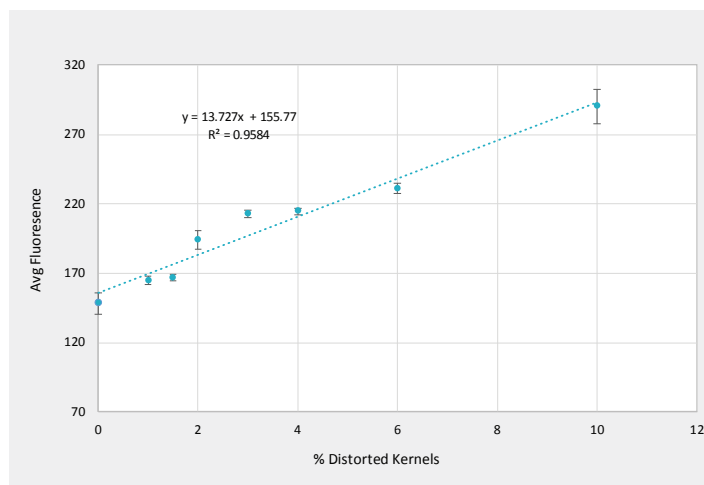**Figure 1** Influence of distorted wheat kernels on flour extensograph Rmax

$$y = 13.727x + 155.77$$
$$R^2 = 0.9584$$

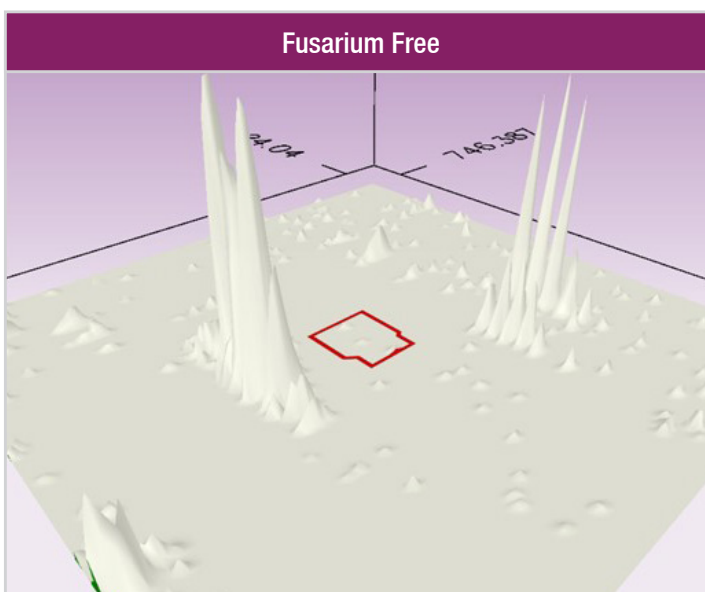**Figure 2** Average flour fluorescence vs wheat FDK% (n= 3 reps)
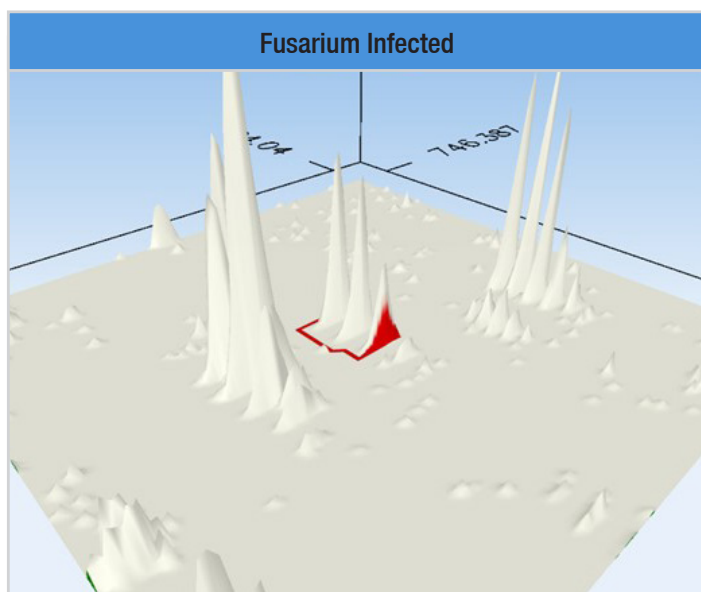
Figure 3 Unique peptides identified due to Fusarium protease activity.

Next steps

We're working with Canadian Grain Commission inspectors to replace current visual assessments with new tolerances for FDK based on the end-use quality effects of Fusarium and the grain safety issues caused by DON.

By exploring new methods to assess both the quality and safety effects of Fusarium damage in wheat, we can address both safety and end-use functionality issues to benefit Canada's grain industry.
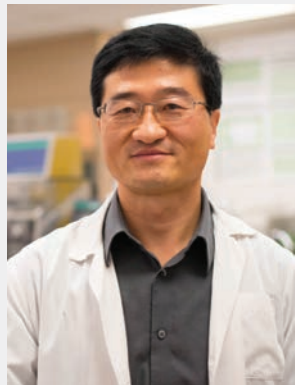
Pulse Research
Dr. Ning Wang

Air-classification: An opportunity for Canadian pulse processing

The global market for plant-based protein ingredients such as those made from pulses is experiencing tremendous growth as populations grow, consumer demographics change, and the cost of animal-derived proteins continues to increase. However, Canada mainly exports whole or split pulses rather than refined ingredients such as flours, concentrates or isolates. This means there is a significant opportunity to benefit Canadian pulse producers by increasing the use of value-added processing for pulses, thereby increasing the demand for Canadian pulses.

Pulses can be processed into flour and further separated, or fractionated, into protein and starch fractions by dry milling, (also called air-classification) or wet milling. Protein and starch fractions could be used as ingredients in food applications. However, new ways of processing pulses means information is needed about the chemical, nutritional, and functional properties of pulse ingredients. Information about how cultivar, environment, and processing affect these properties is also needed.

Air-classification method

To liberate protein and starch, we used fine grinding to grind a sample of peas. Then we used air-classification to separate the finely-ground pulses into protein-rich and starch-rich fractions. Air-classification uses a stream of air to separate fractions according to size and density. We used a laboratory air classifier (pictured below) to:

➤ Investigate the effect of processing parameters such as classifier wheel speed and air flow rate on the composition of air-classified fractions from peas

➤ Optimize the air-classification processing conditions of the laboratory air classifier

Laboratory air classifier for separation of pea flour into protein-rich and starch-rich fractions.

Our results

Our preliminary results showed that classifier wheel speed and air flow rate had significant effects on protein, starch and total dietary fibre content in the protein-rich and starch-rich fractions from pea flour (Figure 1). Increasing classifier wheel speed increased starch content (Figure 1C) of starch-rich fraction, protein (Figure 1B) and total dietary fiber (TDF) content (Figure 1F) of protein-rich fraction, but reduced starch content (Figure 1D) of protein-rich fraction, protein (Figure 1A) and TDF content (Figure 1E) of starch-rich fraction. Increasing airflow rate reduced protein (Figure 1B) and TDF content (Figure 1F) in protein-rich fraction, but increased starch content (Figure 1D) of protein-rich fraction, and TDF content (Figure 1E) of starch-rich fraction.

We established the optimum processing conditions for air-classifying pea flours into protein-rich and starch-rich fractions using the laboratory classifier. Air-classification could double the protein concentration in the protein-rich fraction to 52.3% DM compared to the concentration in pea flour and increase the starch concentration in the starch-rich fraction to 65.5% DM (Table 1). Results also indicated that ash and dietary fiber were mainly concentrated into the protein-rich fraction. Furthermore, we could produce pea flours with different composition by using various combinations of classifier wheel speed and airflow rate.

In conclusion, we found that process variables significantly affected composition of the air-classified fractions derived from peas. We established optimum conditions for separating pea flours into protein and starch fractions using the laboratory air classifier.

Next steps

We will investigate how factors (cultivar, environment, etc.) affect functional properties of protein and starch fractions derived from pulses. This information is important for supporting the increased utilization of pulse ingredients, which will in turn increase the demand for Canadian pulses, benefitting producers.
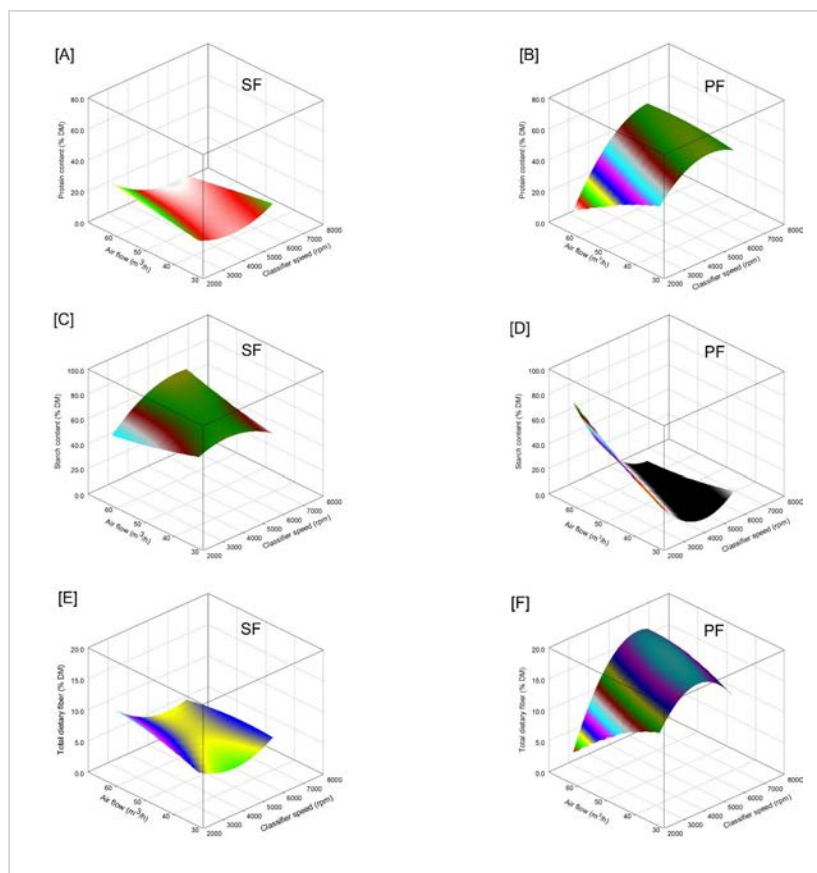

Figure 1 Effect of air classifier wheel speed and airflow rate on (A and B) protein, (C and D) starch, and (E and F) total dietary fiber content of starch-rich fraction (SF) and protein-rich fraction (PF).

Table 1　Composition of raw pea flour, protein-rich and starch-rich fraction produced at optimum processing conditions[1]

Fraction	Protein (% DM)	Starch (% DM)	Ash (% DM)	TDF (% DM)
Raw pea	25.2	49.6	3.5	10.1
Protein-rich fraction	52.3	8.6	6.0	17.8
Starch-rich fraction	12.3	65.6	1.9	6.2

[1]DM=dry matter; TDF=total dietary fiber.

Milling and Malting / Research on Barley and Other Grains

Dr. Marta Izydorczyk

# The effect of variable grain hydration on malt and wort

Enticed by the potential environmental and economic benefits, the malting industry is interested in modifying malting processes to use less water. By steeping malting barley to lower hydration levels, the industry may be able to reduce water use, energy use, and liquid waste.

The goal of our research was to determine the impact of different grain hydration levels during steeping on the content and molecular properties of beta-glucans (β-glucans) and arabinoxylans in malt and wort.

Arabinoxylans and β-glucans in beer production

Arabinoxylans and β-glucans are the major non-starch polysaccharides in barley, and contribute to wort and beer viscosity. Inadequate grain hydration results in incomplete degradation of β-glucans and arabinoxylans. Incomplete degradation can cause processing problems, such as beer haze and slow wort and beer filtration.

Scientific and industry experts in malting had relatively limited understanding of the impact of lower grain hydration on solubilization, hydrolysis or the molecular structure of β-glucans and arabinoxylans remaining in malt and wort.
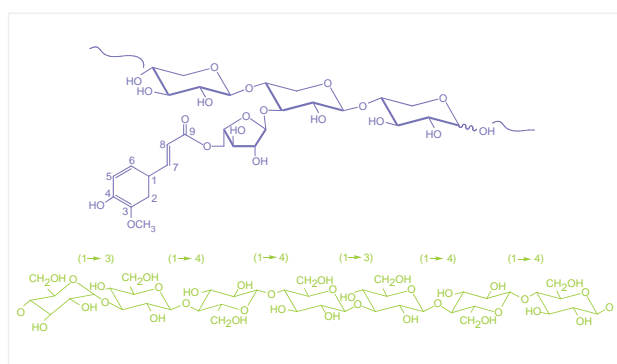
Effect of low (~38%) and high (~46%) grain hydration levels during steeping on the content of β-glucans (BG) and arabinoxylans (AX) in Wort.

The malting bioprocess

Essential to beer production, the malting bioprocess involves steeping, germination, and kilning.

1. Steeping involves hydrating barley grain by repeatedly submerging the grain in water (wet stand) and then allowing it to drain (air rest). The increased moisture and oxygen in the steeped grain initiates germination.

2. During germination, grain produces and releases hormones, synthesizes hydrolytic enzymes to break down the endosperm cell walls and protein matrix, and forms hydrolytic enzymes to break down starch granules.

3. Kilning dries the steeped, germinated grain to a stable malt product, and develops flavour and colour.

Experimental approach

We used CDC Kindersley and CDC Meredith barley samples provided by the Crop Development Centre (University of Saskatchewan) for this study. During malting, CDC Kindersley and CDC Meredith synthesize high and moderate levels of starch-degrading enzymes, respectively. We steeped barley samples to six different grain hydration levels by immersing grain in 13°C water, lifting it from the water, and letting it air rest.

We determined the content and properties of β-glucans and arabinoxylans in malt samples. After we mashed the malt, the polysaccharides remaining in the wort were isolated by precipitation (a method for separating solids from a solution analyzed by high-performance size-exclusion chromatography (Figure 1).

Figure 1
High-performance size-exclusion chromatography (HPSEC, Waters Alliance 2695, Milford, MA) system used to determine the molecular weight and characterize β-glucans and arabinoxylans in wort. Pictured: an online refractive index (Optilab TrEX RI), a multi-angle laser light-scattering (Dawn Heleos II), and a viscosity (Viscostar II) detector (Wyatt Technology, Santa Barbara, CA).
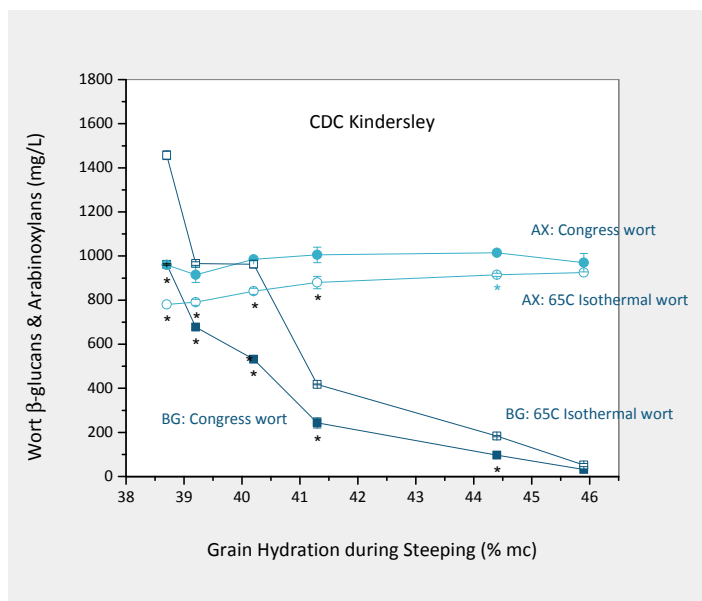

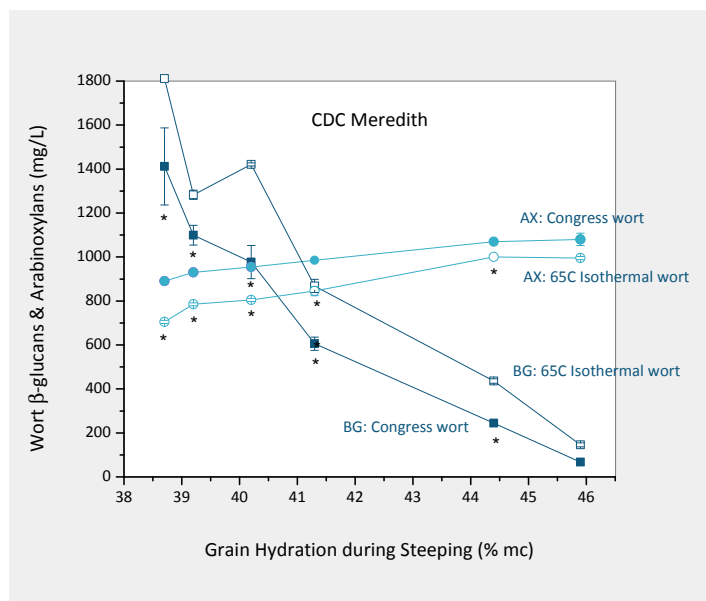**Figure 2** Effects of increasing grain hydration levels during steeping on the content of β-glucans (BG) and arabinoxylans (AX) in wort prepared by Congress mashing and 65°C isothermal mashing for CDC Meredith and CDC Kindersley. Error bars indicate standard deviation. Asterisks (*) indicate statistically significant differences between means for Congress and 65°C isothermal worts at specified levels of grain hydration (p < 0.05).

Results and conclusions

We found that steeping conditions could have a significant impact on the concentration and physicochemical properties of β-glucans and arabinoxylans in malt and wort.

In wort prepared from malted grain hydrated to the lowest level during steeping, β-glucans were dominant. Wort from grain hydrated to the highest level contained more arabinoxylans than β-glucans (Figure 2). Practices that eliminate β-glucans in wort may increase the concentration of arabinoxylans.
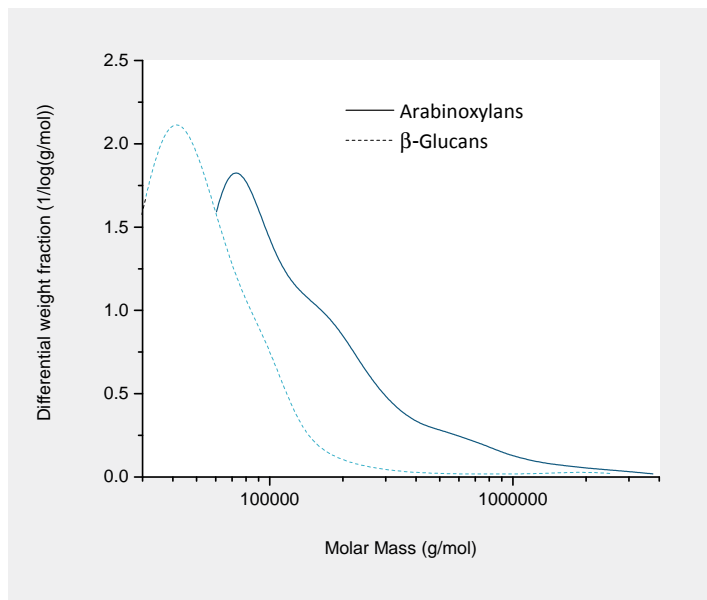

Figure 3 Differential molar mass distribution of polymeric β-glucans and arabinoxylans in 65oC isothermal wort of CDC Meredith prepared from low-hydrated malt (39% mc).

We also found the following:

➤ A relatively small reduction in grain hydration level during steeping significantly increased the concentration of β-glucans in malt (Table 1) and wort (Figure 2)

➤ Some differences between malting barley varieties suggest that reducing the hydration level during steeping may be possible with selected genotypes (Table 1, Figure 2)

➤ Increased grain hydration during steeping caused the concentration of arabinoxylans in malt and wort to increase slightly, indicating that conditions favorable to hydrolysis of β-glucans might cause arabinoxylans to start becoming more soluble (Table 1, Figure 2)

➤ The average molecular weight of wort arabinoxylans was higher than that of β-glucans (Figure 3)

Table 1 Effects of variable grain hydration levels during steeping on the content of β-glucans and arabinoxylans in kilned malt.

Barley variety	Grain hydration during steeping (% mc)	Malt total β-glucans [a] (% dwb)	Malt water-soluble arabinoxylans[a] (% dwb)
CDC Meredith	38.7	1.73 ± 0.03	6.29 ± 0.07
	39.2	1.59 ± 0.04	6.87 ± 0.08
	40.2	1.41 ± 0.02	6.46 ± 0.15
	41.3	0.98 ± 0.05	6.79 ± 0.27
	44.4	0.45 ± 0.00	6.63 ± 0.01
	45.9	0.42 ± 0.03	7.34 ± 0.06
CDC Kindersley	38.4	1.39 ± 0.05	5.82 ± 0.26
	39.3	1.14 ± 0.03	6.24 ± 0.03
	39.9	0.88 ± 0.00	5.85 ± 0.40
	41.4	0.55 ± 0.03	6.67 ± 0.16
	44.2	0.22 ± 0.01	6.38 ± 0.40
	45.7	0.11 ± 0.00	7.12 ± 0.11

Improving grain food safety

Consumers are becoming more aware of food safety risks, and new developments in science and technology are making it possible to detect contaminants more precisely than ever before. Learn how our scientists strive to find new ways to make food safer.

Trace Organics and Trace Elements Analysis

Dr. Sheryl Tittlemier

Better sampling for better results

Testing grain for contaminants can be complicated because some contaminants found in whole grain aren't evenly distributed among kernels in a sample of grain, from a 1,000 gram harvest sample to a 55,000 tonne vessel shipment. This uneven distribution, or heterogeneity, affects the measurement of contaminants in grain because it contributes to variable test results.

This past year, we researched how to adjust sampling and sample preparation to minimize the variability of testing results and, in turn, to minimize the uncertainty of a measurement. Measurement uncertainty is the range in which the true value of a measurement is expected to be. When we narrow this range, we decrease the uncertainty.

What does heterogeneity look like?

An easy way to visualize heterogeneity is when kernels of a grain such as wheat, rye or barley, all of which contain gluten, are found in a gluten-free grain such as flax that's to be used in the production of gluten-free foods. This is a less complex scenario (Figure 0, left), because the seeds either contain gluten (wheat, rye, or barley) or don't (flax).

A more complex example of heterogeneity is the presence of mycotoxins such as deoxynivalenol (DON) in wheat. The amount of DON in one wheat kernel can vary greatly compared to the next (Figure 0, right). In one of our research projects, we saw that DON varied from less than 0.5 ppm (parts per million) up to 260 ppm in single wheat kernels that had various degrees of visible Fusarium damage (Figure 1).
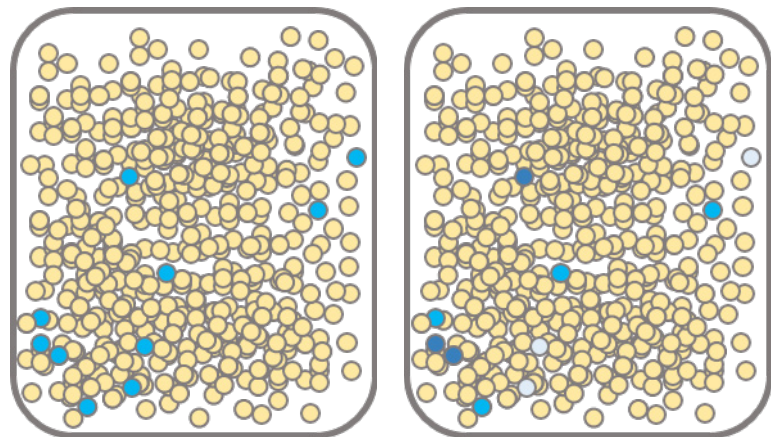

Figure 0 Illustrations of simple (left) and complex (right) heterogeneity of contaminants in grain.

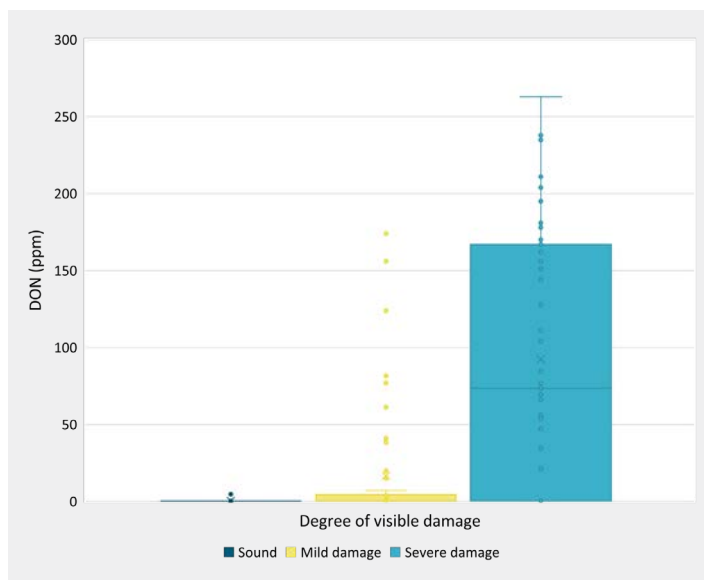Ground flax for the analysis of gluten contamination.

Figure 1 Observed range of concentrations of the mycotoxin deoxynivalenol (DON) across different degrees of visible Fusarium damage.
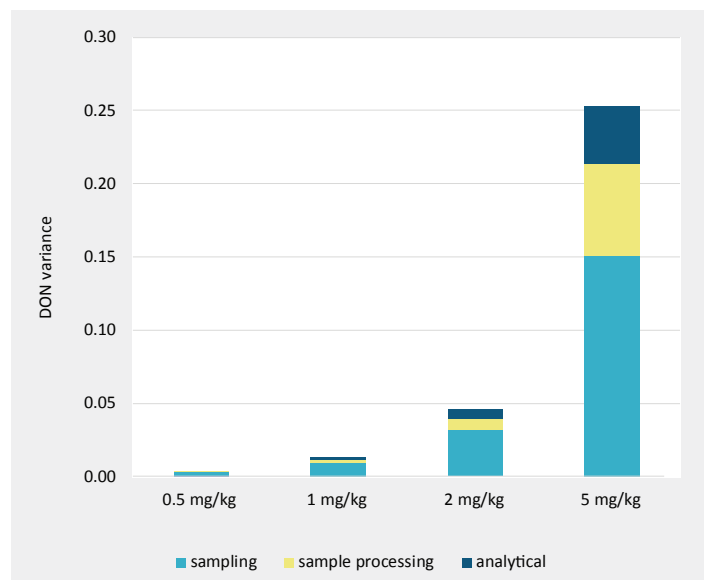

Figure 2 Relative size of contributions to the variability of DON test results from the different stages of the analysis procedure.

Analyzing gluten contamination

We evaluated two sample preparation schemes with the aim to minimize the effects of sample heterogeneity when analyzing gluten contamination in oilseeds, pulses and oats. As little as 4 to 5 kernels of wheat could contaminate a 1 kg sample of gluten-free grain, exceed regulations, and affect gluten-intolerant or gluten-sensitive consumers. Therefore, accurate and precise analysis of gluten is important.

Investigating DON testing variability

We analyzed 16 lots of wheat that contained 0.26 to 25 ppm DON. In collaboration with researchers from North Carolina State University, we divided, prepared and analyzed the wheat lots. We followed an experimental design that incorporated repeated testing so we could characterize different sources of variability. We performed almost 500 DON analyses for this project.

Results (Figure 2) showed that taking a larger sample of whole grain did the most to decrease the variability of DON results. After that, grinding more grain and grinding to smaller particle sizes both decreased variability. The test method used to measure DON in wheat had the least effect on variability of results.

We'll use the results from this project to develop a model for which users can develop sampling plans in order to balance the risks of measurement uncertainty with the amount of testing required to minimize those risks.

In our laboratory, between the two schemes, we compared:

➤ the size and extent of grinding sample in the laboratory (Photo 1)

➤ the method of sub-sampling ground grain

➤ the mass of sample tested

We saw the best improvements in variability of gluten crossing with grinding more grain prior to sub-sampling.

Acknowledgements

Microbiology

Dr. Tom Gräfenhan

Investigating bacterial pathogens in grain commodities

Food safety is important to consumers and is one of the food industry's top priorities. National regulators around the globe, as well as key members of the food industry, demand better and more effective monitoring so that health risks are managed more proactively. A number of potential threats, either chemical or microbiological in origin, can arise at specific points throughout the grain supply and processing chain. By assessing microbial contaminants in grain commodities, the Canadian Grain Commission's Microbiology Program plays an important role in grain food safety.

Food-borne bacteria can have an immediate effect on human health, causing sickness or even death within days. In the past, low-moisture foods such as ready-to-eat grains or milling wheat were considered lower risk commodities. Lack of sufficient water activity or optimal temperatures usually prevent the growth of pathogenic bacteria. During food preparation, however, conditions become more conducive to bacterial growth, occasionally to levels of food poisoning.

Pulsifier equipment uses high frequency vibrations to homogenize a whole grain sample releasing surface-attached microbes into suspension.

For microbiological analysis, grain samples are weighed in sterile plastic bags and soaked in selective enrichment broth.
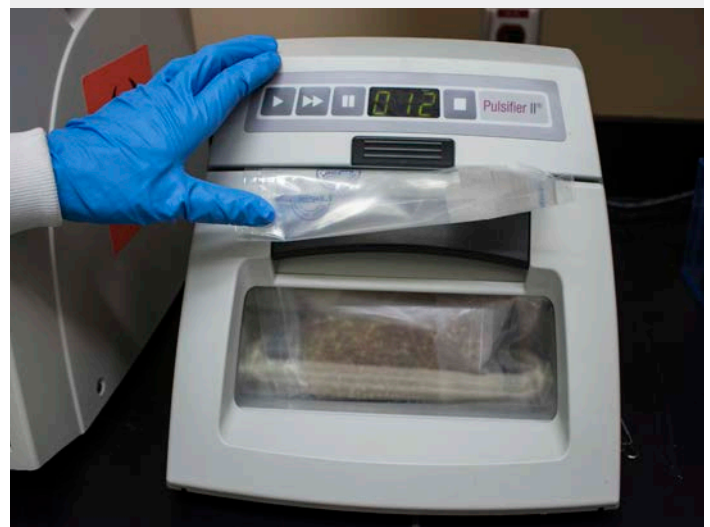
Methods

In 2017, 30 confirmed cases of food poisoning caused by verotoxigenic *Escherichia coli* bacteria were traced back to wheat flour sold across Canada. Consumption of raw dough was identified as potential source of infection. In collaboration with the Canadian Food Inspection Agency (CFIA), we investigated the occurrence and frequency of pathogenic bacteria such as *Listeria, Salmonella* and *E. coli* in grain samples.

To identify and characterize these important pathogens, we use traditional microbiological methods, bio-molecular diagnostics and whole genome sequencing. We use similar methods to analyze raw grain as those developed by the CFIA for testing flour samples. This analytical work often requires using containment laboratories to protect our personnel and samples for safety and quality assurance.

During enrichment, maintaining temperature and conducive conditions in incubators is critical for bacterial growth.

Antibiotic solution is added to samples inhibiting the growth of non-priority bacteria.

On selective media plates, pathogenic bacteria can be identified by direct observation of distinct characteristics such as colony colour.

Bacillus cereus

After the 2017 harvest, we tested over 500 wheat and 100 flax samples for human-pathogenic bacteria. None of the grain samples were contaminated with any of the priority pathogens we screened for. However, we did regularly detect the spore-forming microorganism, *Bacillus cereus*, which is commonly associated with grain.

Bacillus cereus is often found in soil, dirt and water, or on plants and decaying organic matter. Interestingly, its sister species, *B. anthracis*, is better known because it's the infamous agent of anthrax. Genetically, the two bacteria are closely related and belong to the *B. cereus* group.

Some members of this group can also cause food poisoning and infections in humans. Heavy contamination by toxigenic strains of *B. cereus* in foods such as meats, milk, cheese, spices, rice and pasta can cause vomiting or diarrhea. Our recent study revealed a low frequency of *B. cereus* strains that actually produce the emetic toxin. Less than 5% of the isolates from grain samples showed a propensity to form cereulide, a depsipeptide that causes vomiting.

Collaboration in food safety

With our partners at Genome Quebec, the National Microbiology Laboratory and other reference labs, we've taken a collaborative approach to grain food safety. For instance, our partners provide reference material, high-throughput sequencing and high-performance computing that are essential to our pathogen research and surveillance program. Whole genome data and bioinformatics analysis have become the new standard for bacterial typing, molecular epidemiology and pathogenomics. Genomics-based methods are used to not only identify and characterize food-borne pathogens but also to trace the source responsible for microbial contamination. Whole genome sequencing and comparative data analysis provide new pathogen information with better resolution and enhanced reproducibility. Therefore, our laboratory is creating additional capacity to anticipate and mitigate the risk of potential disease outbreaks or market barriers for grain shipments.
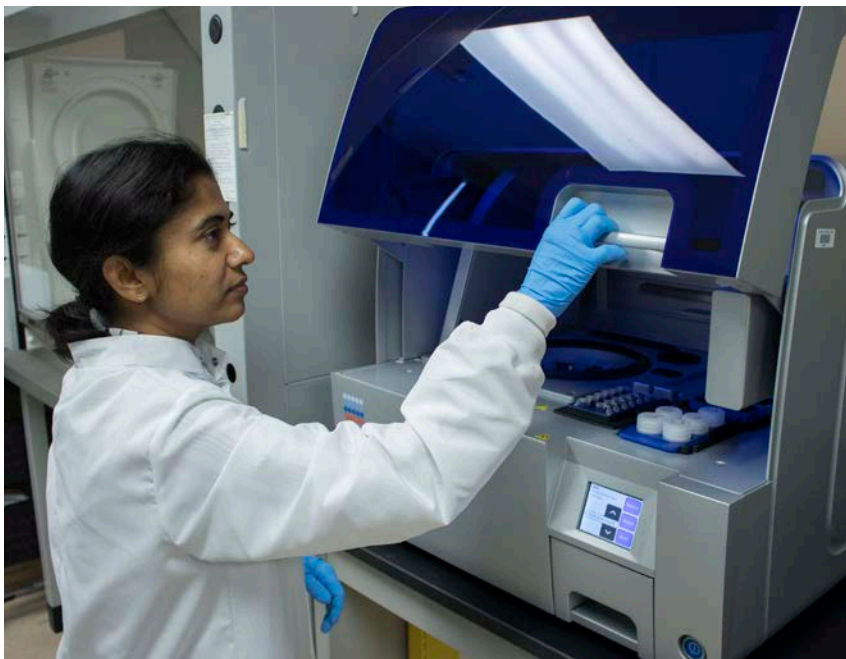
An automated nucleic acid extraction system is a time-saving and effective tool to isolate DNA from bacteria.
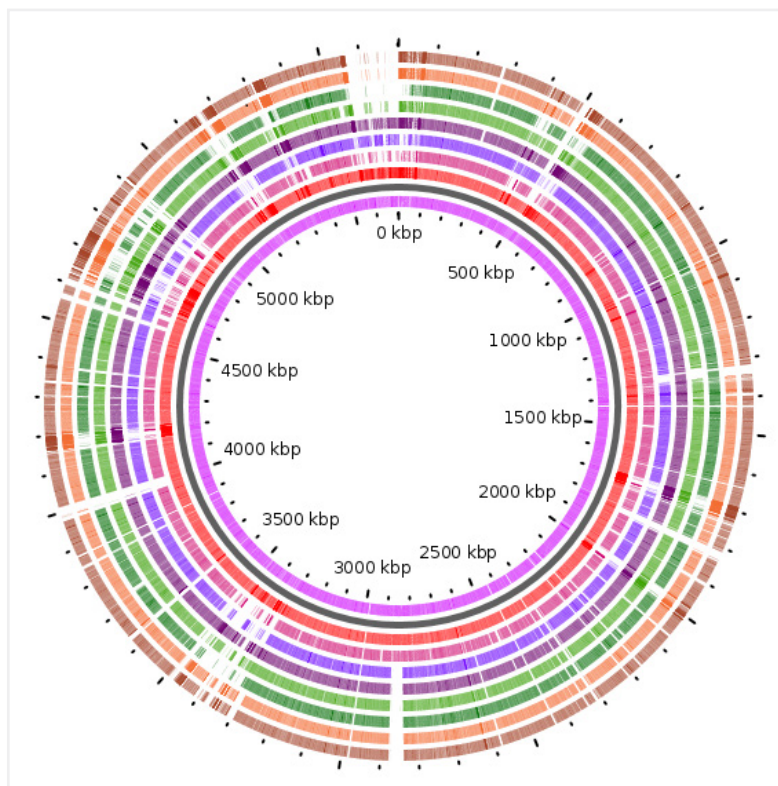
Circular map for comparison of bacterial genomes highlighting genetic regions that are common to nine E. coli cultures sequenced.

Bio-molecular tests such as real-time PCR are performed to screen grain samples for priority pathogens such as Listeria, Salmonella or verotoxigenic E. coli.

Variety identification

From restoring market access to solving crime

In a rapidly evolving grain sector, the ability to quickly and precisely identify the genetic makeup of a grain kernel has become an essential tool for resolving a variety of issues. From proving that a specific variety of wheat isn't present in the Canadian grain handling system, to identifying hundreds of bushels of stolen canola, the Canadian Grain Commission's scientists have played a vital role in addressing issues arising from varietal identity, trait identity and traceability.

Variety Identification Research and Monitoring

Dr. Daniel Perry

Evolution of wheat variety monitoring

Over the past three decades, the role of variety monitoring in Canada's wheat quality assurance system and the technology used to do it has evolved considerably.

When variety monitoring was first implemented, kernel visual distinguishability (KVD) was a registration requirement for new western Canadian wheat varieties. KVD meant that all varieties in a class looked similar, which facilitated class segregation and grading. However, unregistered varieties could look like registered varieties, potentially undermining quality. Variety monitoring to confirm that the correct varieties were present provided confidence that wheat would perform as expected, protecting the reputation of Canada's wheat for consistent quality.

A technician identifies wheat varieties by comparing the gliadin banding patterns with reference standards.

1990s

Protein electrophoresis

Beginning in the 1990s, the Grain Research Laboratory used protein electrophoresis for wheat variety monitoring. Gliadin proteins were extracted from crushed individual kernels using alcohol, separated by passing through an acrylamide gel under an electric current, and stained to reveal banding patterns. Technicians identified varieties by comparing these patterns to reference standards, a process that required considerable skill and time. Many wheat varieties had unique gliadin patterns, but some could only be narrowed down to a group of possibilities. In cases where the possibilities included both eligible and ineligible varieties, we needed higher resolution methods.

2005

DNA analysis

We began regularly supplementing protein electrophoresis with DNA analysis in 2005. When we needed a more precise identification for a particular kernel, we used the residue left over after protein extraction to determine a DNA profile for that kernel. We developed profiling methods based on variable regions of DNA known as microsatellites. We amplified specific sets of microsatellites from each kernel's DNA using polymerase chain reaction (PCR). The resulting DNA fragments were separated by size in an automated DNA analyzer equipped with a fluorescence detection system to create gel images. Technicians still had to match the resulting banding patterns manually, but the resolution among varieties was much better.

Placing a gel cassette in an automated DNA analyzer in preparation for microsatellite DNA analysis.
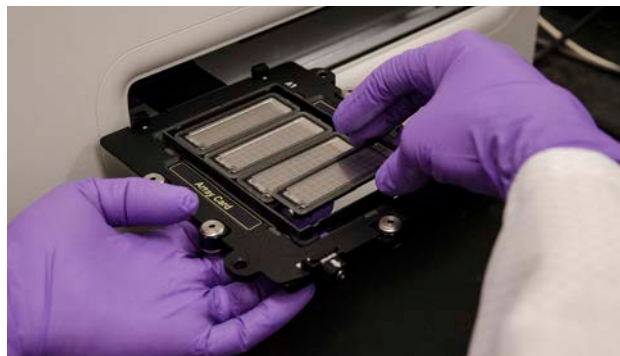
Placing OpenArray plates in an instrument for analysis. Up to four plates containing the DNA of 384 individual kernels of wheat can be analyzed at once.

KVD was removed as a requirement for variety registration in 2008. The Canadian grain industry began requiring producers to declare that they were delivering an eligible variety, and the Canadian Grain Commission increased variety monitoring. Developing and registering new wheat varieties takes years, so the full impact of eliminating KVD wasn't felt immediately. One of the first significant challenges occurred in 2015 when several high-yielding, lower-protein wheat varieties from the United States were registered in Canada. These varieties were ultimately designated to the new Canada Northern Hard Red class, but looked like Canada Western Red Spring.

In 2018, the wheat class modernization initiative decisively ended the KVD era by moving 25 Canada Western Red Spring and 4 Canada Prairie Spring Red varieties to the Canada Northern Hard Red class. We now rely on laboratory testing for variety monitoring more than ever.
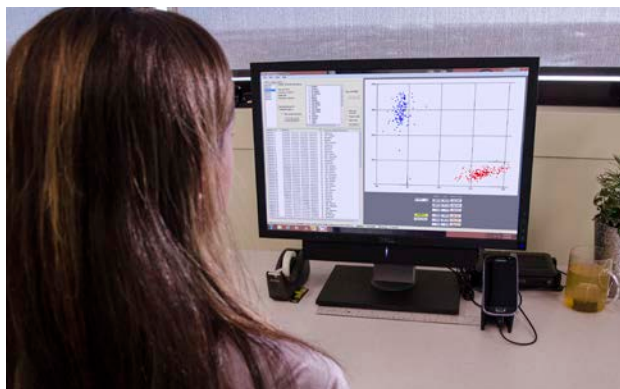
Reviewing the results of automated variety calling performed by our VID Inspector software.

2011

OpenArray

In 2011, we began supplementing protein electrophoresis with a different type of DNA analysis that targets specific positions in the DNA where we know there are small differences among varieties. Differences might be a single letter altered in the DNA code, or a small piece of DNA missing or added. We assess these targets using OpenArray genotyping technology. On one OpenArray plate, about the size of a microscope slide, we can analyze 32 DNA targets in each of 96 kernels of wheat. Fluorescence readings are taken at two wavelengths. The relative intensities vary depending upon the DNA sequences present.

We developed data analysis software that automatically interprets the OpenArray readings, builds a profile for each kernel, and searches for a match in our variety database. This eliminated the need to manually interpret banding patterns, which was the most labour-intensive part of protein electrophoresis and microsatellite analysis. By the end of 2015, we were exclusively using our OpenArray method for wheat variety identification.

During the 2017-2018 crop year, we analyzed about 82,000 individual kernels of wheat. For 2018-2019, that number could nearly double as we monitor the movement of 29 wheat varieties out of their former classes.

Future

Safeguarding quality into the future

In an evolving global market, variety monitoring plays a major role in ensuring that grain buyers can confidently rely on the quality of Canadian wheat. We'll continue to evaluate and work with promising new technologies so Canada's grain quality assurance system is prepared to respond to any future challenges.

Grain Biotechnology Research

Dr. Tigst Demeke

Detection and management of genetically modified crops

In 2017, 189.8 million hectares of genetically modified (GM) crops were grown globally. Soybean, maize, cotton and canola are the most adopted biotech crops, grown in 24 countries. In 2017, 77% of soybean, 80% of cotton, 32% of maize and 30% of canola grown worldwide were genetically modified (source: International Service for the Acquisition of Agri-biotech Applications).

The unplanned presence of unapproved genetically modified (GM) events in shipments is a challenge for the grain and oilseed industry. If a specific GM event has not received regulatory approval in an importing country, that country may not accept shipments that contain that GM event.

For example, the FP967 GM flax event was approved in Canada and the United States, but was not approved in importing countries such as Japan and member countries of the European Union. The European Union has a zero tolerance policy for shipments that contain any FP967 GM flax. Testing for the presence of FP967 GM flax event started in 2009 and continues to this day for shipments to the European Union.

Wheat sample for grinding

Grinder

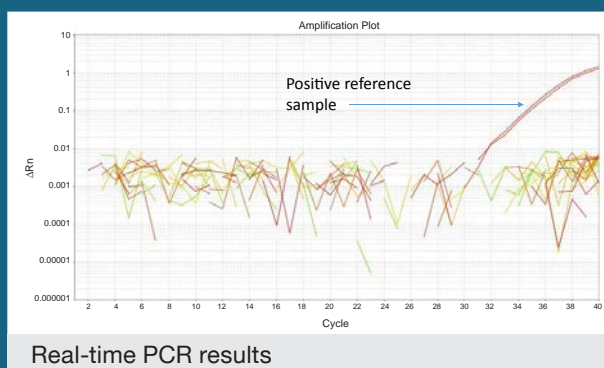Real-time PCR results

Figure 1 Steps involved in testing of wheat vessel shipments for the presence of MON71200 GM wheat event. DNA is extracted from a portion of the ground sample and used for PCR. The PCR result shows signal from the positive reference sample only. The samples tested did not produce any signal showing the absence of MON71200 GM event.

Genetically modified wheat has not been approved for commercial production anywhere in the world. However, since the late 1990s, field trials of GM wheat have been conducted in Canada and the United States. Monsanto was close to commercializing Roundup Ready wheat in 2004, but made a decision to halt commercial production because of concerns from major buyers of North American wheat. In the United States, isolated incidents of GM wheat were discovered in Oregon (2013), Montana (2014) and Washington State (2016). The MON71800 GM wheat event was discovered in Oregon and Montana, while the MON71700 GM wheat event was discovered in Washington State. These were isolated incidents and the United States grain handling system was not affected.

On June 14, 2018, the Canadian Food Inspection Agency (CFIA) publically announced that a small number of glyphosate-tolerant wheat plants (MON71200 GM event) had been found growing alongside an access road in southern Alberta. We worked with CFIA and other federal and provincial partners on the best way to handle the issue.

Proving Canadian wheat shipments are GM free

Testing carried out in our laboratory and at the CFIA confirmed that the MON71200 GM wheat event wasn't present in Canada's grain handling system. We started testing wheat vessel shipments for this event in June 2018. We sampled and tested all wheat shipments exported from Canada via terminal elevators, and provided a letter of analysis to clients upon request.

Preparation of wheat DNA for MON71200 GM event test.

By the end of December 2018, we had tested a total of 491 wheat vessel shipments and hadn't detected the MON71200 GM wheat event in any of the samples analyzed. These results conclusively show that Canadian wheat is free from the MON71200 GM wheat event.

Test method

Our testing involves grinding a 400 g (approximately 10,000 wheat grains) official wheat cargo sample (Figure 1). We carry out duplicate DNA extractions on a small portion of the ground sample. We use the DNA for the detection of the MON71200 GM wheat event employing polymerase chain reaction (PCR). We use event-specific PCR method provided by the CFIA for testing. Event-specific PCR method provides accurate detection of the MON71200 GM wheat event.

When we test wheat vessel shipments for the presence of MON71200 GM wheat event, we extract DNA from a portion of the ground sample and use the PCR.

Ground wheat sample

DNA extraction kit

Instrument for measuring DNA amount

Real-time PCR instrument

Oilseeds

Dr. Véronique Barthet

Using chemical analysis profiles to identify rapeseed samples

The case

Searching for stolen grain, the Royal Canadian Mounted Police recovered canola samples from producer bins and from loads delivered to grain elevators. The chemical analyses of the samples provided by the RCMP showed that two samples were statistically chemically similar. However, no study had determined if and how chemical similarity could be used to determine if different canola samples are from the same source.

What is the probability that two samples with the same chemical profile came from different sources? We needed to assess whether we could use chemical profiles to classify canola samples of unknown identity correctly.

The investigation

DNA evidence isn't enough to confirm that two grain samples are from the same bin because the same grain varieties are grown in several places in Canada. Both genetics (the variety) and weather and soil conditions govern canola quality parameters.

We developed a program for grouping random samples based on pair-wise comparison to every observation in the database, across each quality parameter. We estimated the variance in matching random samples to known identities based on similarity in their parameter values. We obtained the variances allowed on each parameter from previous statistical analyses, based on the repeatability and reproducibility of the test. These variations (standard deviations) included error due to the analysis and error due to sampling. We used the known variance in analyses for each parameter to pair similar samples. For example, a sample with oil content of 43.5% and a sample with oil content of 43.2% were considered identical because the variance was ±0.3% for the oil content analysis.

We created a database of 22,277 canola samples to allow the pair-wise comparison, including harvest year, grade, variety, oil, protein, glucosinolates, chlorophyll, oleic acid, linolenic acid, saturates and iodine value (Table 1). The fixed variables used were: (a) grade, (b) class, and (c) variety. Using these

parameters, we found 5 to 6433 potential matching pairs. We then used the samples' chemical composition to further pair potential matches.

Narrowing the matching pairs by protein content identified just one pair of matching high erucic acid (HEAR) samples, probably because the database contained only 235 HEAR (high erucic acid rapeseed) samples. This pair was chemically similar and represented 0.85% of HEAR samples.

When oil content and glucosinolate content were added, the number of possibilities was greatly reduced and some matching pairs were identified. However, it was not possible to match some of the LEAR (low erucic acid rapeseed) and LowLin (low linolenic acid) B. napus samples completely. We finally separated the matching pairs by partial fatty acid composition (oleic acid, linolenic acid and saturates content).

We found five pairs of canola samples of similar quality and known identified variety, meaning that only 0.045% of the samples of known variety from our database were chemically similar. The matching samples were all grown in the same year and geographically near each other, except for one pair grown in different provinces.

Another 33 pairs – 0.30% of database samples – were chemically similar samples of unknown variety. Of those 33 pairs, three pairs were grown more than four years apart; we supposed these three pairs were likely not the same variety. Only 30 pairs of samples could be added to the previous five matching pairs.

Results in court

The probability of two samples with the same chemical composition being from two different sources is extremely low. Out of 22,277 samples, 35 sample pairs of similar quality and identical class, sub-class, grade and variety were found. At best, 0.30% of the canola samples grown in Canada are identical based on class, subclass, variety and chemical composition.

We provided documents that were filed in court and helped the Crown successfully pursue its case. The individual was eventually convicted of possession of stolen property.

Table 1 Database parameters used for the pair–wise comparison. Data collected from 2000 to 2010 Canadian Grain Commission Harvest Survey.

Variable	Min	Max	Min	Max	Min	Max
Class	B. napus		B. rapa, B. napus & B. juncea B		B. napus	
Subclass	HEAR		LEAR		LowLin	
N subclass	235		20468		2221	
N Variety	3		287		65	
N per variety	39	138	1	1115	1	193
N Unknown variety	58		6436		278	
Oil (%, 8.5% moisture)	38.0	50.0	31.4	52.2	13.1	51.5
Protein ((%, 8.5% moisture)	17.3	29.2	14.5	31.5	13.8	30.5
Glucosinolates (umol/g seed)	3.1	18.1	0.0	38.2	1.0	28.2
Chlorophyll (mg/kg)	0.0	128.3	0.0	211.6	0.0	161.0
Oleic acid (% in oil)	9.9	38.4	0.0	74.7	57.6	79.3
α-linolenic acid (% in the oil)	6.7	12.7	0.0	16.6	0.2	12.8
Total Saturates (% in the oil)	4.5	5.9	0.0	9.5	5.4	8.2
Iodine Value (units)	96.6	112.2	0.0	132.0	90.7	114.8

HEAR = high erucic acid rapeseed   LEAR = low erucic acid rapeseed   LowLin = low linolenic acid

Since the early 2000s, canola has been a target for crooks, with prairie farmers losing tens of thousands of dollars when thieves help themselves to the contents of an unsupervised grain bin. These brazen burglars can steal a few hundred bushels out of a grain bin, deliver it to a grain elevator and walk away with a cheque. Farmers who don't check their bins regularly may not be sure if canola was actually stolen or when the theft occurred.

Even when a farmer reports stolen canola to the RCMP, proving that canola came from a certain person's bin is very difficult. In 2012, the RCMP reached out to Dr. Barthet to see if she could prove that hundreds of bushels of stolen canola actually came from a complainant's bin.

Thanks to her research, Dr. Barthet was able to prove that the stolen grain sold at a primary elevator was a match for a victim's canola.[1]

[1] Mallory Procunier, "When They Reap What You Sow: Cracking Down on Canola Thefts in Rural Canada," *Gazette / Royal Canadian Mounted Police* Vol. 74, No. 2, 2012, p. 14.