# Evaluation of Incidence and Prevention

# of

# Blackleg and Bacterial Ring Rot

**Potato Growers of Alberta**
**Progress Report 2007/08**

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**Summary**

Sensitive diagnostics have been developed that are capable of detecting trace levels of the blackleg and bacterial ring rot pathogens. The procedure works on extremely small samples of only a few milligrams, may be used to examine any sample including soil, and results can be available within only a few hours. The procedures are quantitative facilitating the estimation of pathogen levels in seed or soils before planting and are capable of differentiating between strains with different characteristics such as aggressiveness and symptom expression. Results from over 200 samples show few occurrences of the pathogen causing bacterial ring rot but an increasing incidence of blackleg samples. Several virulent soil probiotics that aggressively attack blackleg and bacterial ring rot pathogens have been isolated and are being offered for application as a seed treatment that prevents blackleg and ring rot. Greenhouse and field trials have been established for the evaluation of disease symptom expression models in potato varieties, characterization of the diagnostics, and determination of the most effective application parameters for the prevention measures. Pesticide Management Regulatory Agency has provided for application of the proactive seed treatments and producers are encouraged to continue to submit diseased samples for confidential evaluation and thereby assist in characterizing the diagnostics and prevention strategies. Agriculture and Agri-Food Canada continues to match the Potato Growers of Alberta contributions for this project through support of a competitive Matching Investment Initiative application. This project is now entering the third and final year.

**Background**

Blackleg and tuber soft rot of potato are caused by pectolytic gram negative *Erwinia* species. These diseases are found wherever potatoes are grown. The incidence and severity of blackleg appears to be increasing in western Canada potato producing areas. Blackleg is favoured by cool wet soils at planting and spread through seed, irrigation, and insects. Blackleg can cause severe yield losses and symptoms may appear at any stage of plant development. Symptoms progress from a decaying seed piece to lesions extending from the base of the stem into the canopy. Several species of *Erwinia* are known to cause disease but many factors contributing to the disease are poorly understood. Additional information on the transmission, detection, and control of blackleg would improve yields and quality.

Bacterial ring rot has plagued the potato industry and is a zero tolerance pathogen. It is caused by a gram positive tuber-borne bacterium, *Clavibacter michiganenesis* subsp. *sepedonicus*. The bacterium can overwinter in potato debris, may reside in other hosts such as sugar beets, can be spread by insects, and survives on equipment for up to 5 years. Symptoms vary amongst potato varieties and environmental conditions. Unfortunately, the identification of a single infected tuber can result in decertification, sometimes bankruptcy, and negatively impacts trade. Our understanding of bacterial ring rot is still quite limited and alternatives for detection and control are required.

Probiotics have recently emerged as an important tool in the control of human and animal bacterial diseases. Probiotics are nature's control mechanism, naturally occuring for each bacterium, and represent a cost-effective prevention strategy for blackleg and bacterial ring rot. Diagnostics that identify pathogen sources and strains and disease control strategies based on management and biocontrol, should reduce the occurrence of blackleg and bacterial ring rot.

**Objectives**

1) Develop sensitive diagnostic tests that reliably detect the pathogens causing blackleg and bacterial ring rot. Assays will be applied to determine sources, vectors, and pathogen strain distribution in soils selected for potato production.

2) Characterize the pathogen populations causing blackleg and bacterial ring rot in Alberta. Forensic samples will be obtained from diseased tissues, soils, equipment, storages, and collections to determine virulence, aggressiveness, and other characteristics such as transmission.

3) Develop strategies to control of blackleg and bacterial ring rot. This will involve a management approach based on the diagnostic monitoring information, the screening of AAFC advanced lines and commercial varieties for symptom expression, and seed and soil phage biocontrol amendments.

4) Improve the competitiveness and sustainability of producers and processors by advancing our understanding of these diseases to curtail their occurrence and improve yield and quality.

## Materials and Methods

1) Pathogen identification, and isolation: Industry, CFIA, and collaborators are assisting in the collection of diseased samples and blackleg and bacterial ring rot pathogen identification/isolation. Additional pathogen populations will be obtained from existing regional, National, and International culture collections for comparison.
2) Detection and quantification: Sensitive pathogen-specific polymerase chain reaction (PCR) assays have been developed to detect and quantify nucleic acid from each pathogen. Universal primers designed for highly conserved rDNA sequences have proven effective for reliable identification of the pathogens. Testing is examining various sources of the pathogens including field soil, potential vectors, alternative hosts, equipment, storages, and potatoes.
3) Strain characterization: AAFC has developed PCR assays of genetic variability within each pathogen to determine strain populations. Hypervariable intergenic regions are capable of distinguishing even small variations in pathogen populations. PCR amplifications are performed under stringent conditions and amplified products cloned and sequenced. Sequence comparisons and analyses are performed with various available software programs such as Mulialign.
4) Disease management: Management practices and pathogen threshold values will be evaluated to determine strategies to control pathogen reservoirs, vectors, and minimize disease losses. Advanced lines from the AAFC and commercial cultivars are being screened with aggressive strains of blackleg and bacterial ring rot pathogens in storage, greenhouse, and/or field trials for symptom expression. Soil, storage, and seed treatments, irrigation, and crop rotations will be assessed to identify and recommend strategies to reduce disease. Phagetherapy with isolated natural viruses from this study for blackleg and bacterial ring rot will be evaluated as a cost-effective biocontrol to prevent disease.

## Results and Discussion

This project commenced in the spring of 2006. Agriculture and Agri-Food Canada approved an application to match the Potato Growers of Alberta cash and in-kind contributions. Excellent progress has been made in both the development of diagnostics and the isolation of aggressive virulent probiotics for blackleg and ring rot. Producers are encouraged to continue submitting diseased samples for confidential evaluation and thereby assist in characterizing the diagnostics and prevention strategies.

Isolates and Diagnostics

Industry, the Canadian food Inspection Agency, and collaborators assisted with the collection of diseasesd blackleg and BRR samples for pathogen identification and isolation. Over 200 samples of blackleg and BRR from North America were collected for development of diagnostics, characterization, and prevention strategies. Cultures were evaluated for aggressiveness and suitability in greenhouse and field trials (Figure 1). Several of the most aggressive isolates selected for screening advanced lines and varieties for symptom expression and eventually effectiveness of diagnostic and prevention measures (Table 1). Additional pathogen strains will be obtained from existing regional, National, and International culture collections for comparison.

**Figure 1.** Agricuture and Agri-Food Canada Stavely Substation 2007 field plots for screening advanced lines, diagnostics, and biocontrol products to BRR and blackleg. This is the only site in Canada for field BRR analysis. Some advanced lines and varieties show no disease symptoms, however, most lines show some degree of foliage and tuber symptoms but this is clearly influenced by the environment and weather.

**Table 1.** Disease ratings for bacterial ring rot from the hand planted and harvested 2007 field plots at the AAFC Stavely Substation. The BRR model correctly predicted pronounced symptom expression this year.

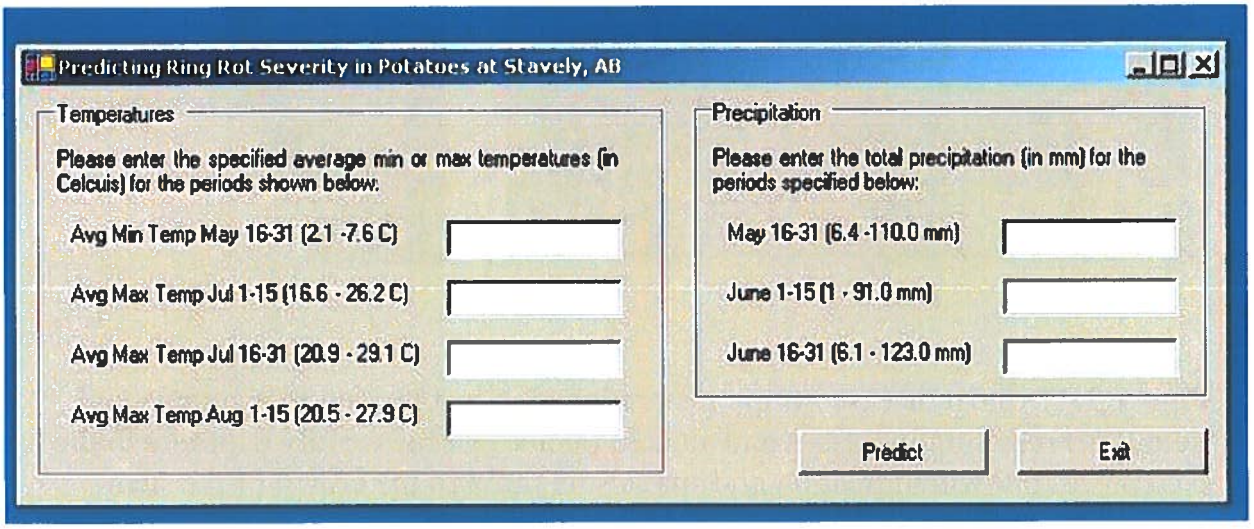
| **Foliage** | **Mean** | **S.E.** | |
|---|---|---|---|
| Alpha/R | 0.00 | 0.00 | 0 - no visible symptoms |
| Russet Burbank/R | 1.80 | 0.20 | 1 - wilt only on lower leaves |
| Norland/R | 4.47 | 0.22 | 2 - wilt/chlorosis on lower leaves |
| FV11579-3/R | 3.07 | 0.23 | 3 - wilt to the top of plant |
| FV12228-5/R | 4.00 | 0.31 | 4 - wilt/chlorosis to top of plant |
| FV12272-3/R | 2.93 | 0.25 | 5 - plant dead |
| V0379-2/R | 0.47 | 0.17 | Controls uninoculated |
| **Tubers** | | | |
| Alpha/R | 0.9 | 0.9 | Tuber Rating (30 max) = |
| Russet Burbank/R | 3.7 | 0.1 | [(Rot Tuber/Total) x 3 |
| Norland/R | 4.4 | 1.0 | + (Surface-Internal/Total) x 2 |
| FV11579-3/R | 6.6 | 2.5 | + (Internal Only/Total) x 1)] x 10 |
| FV12228-5/R | 7.8 | 1.0 | |
| FV12272-3/R | 8.8 | 1.4 | |
| V0379-2/R | 0.1 | 0.1 | |

Results indicate that cultural practices may be contributing to the increased occurrence of blackleg (Figure 2). Most seed has low levels of the *Erwinia* species causing blackleg according to Canadian Food Inspection Agency results but the incidence is too low to produce disease under typical circumstances. However, management practices such as fall irrigation provides a moist cool environment during seed planting and conditions conducive to the occurrence of blackleg. This observation will be further investigated and the benefits of the probiotics in eliminating all traces of the pathogens evaluated.

**Figure 2.** Typical disease symptoms produced in by blackleg in a commercial field (left) resulting in misses and stunted plants. Tissue rapidly degrades in the seed piece following infection and spreads up through the crown of the stem (right).

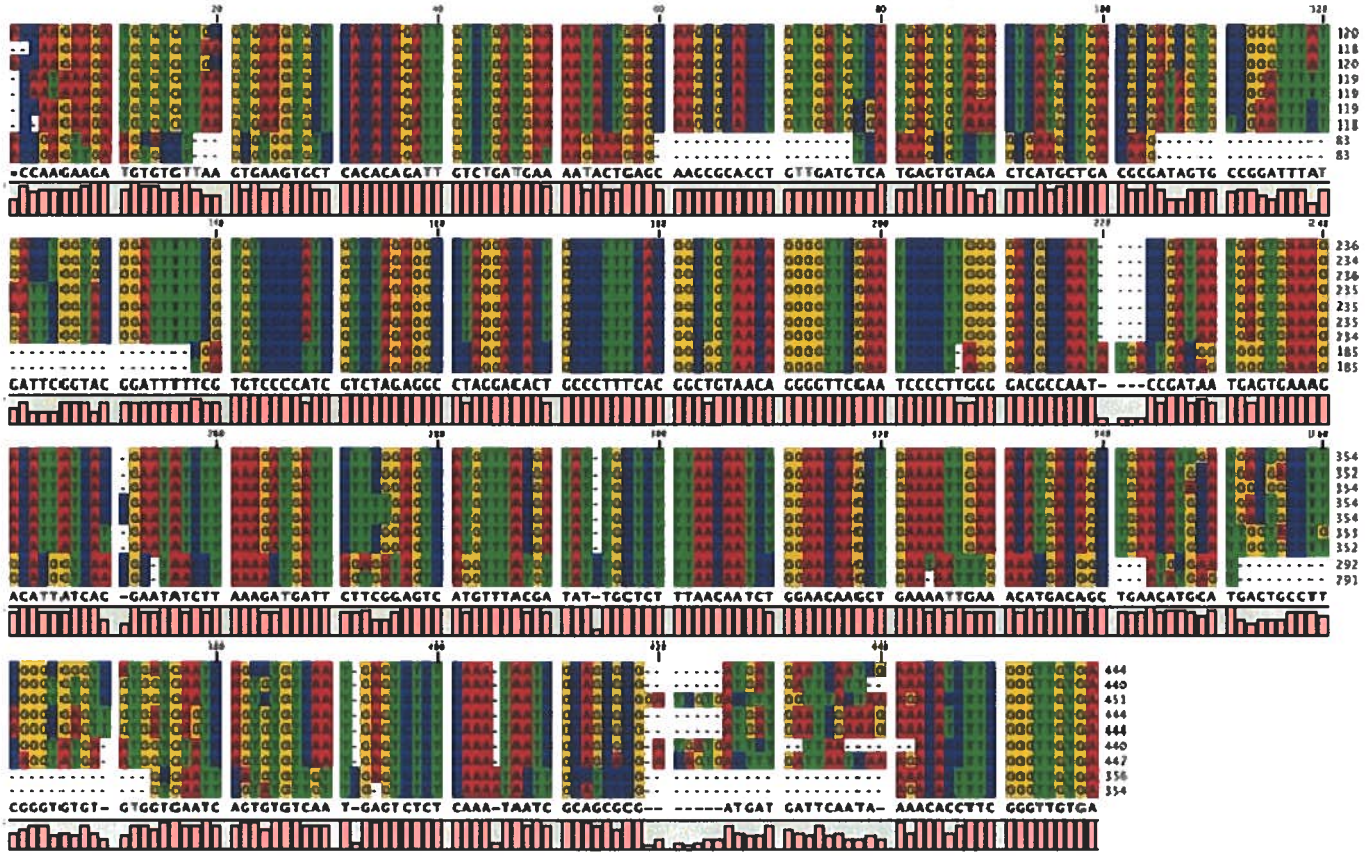
An executable program developed from 12 years of BRR data generated by neural analysis of the 384 genotypes evaluated at the only Canadian field testing facility, facilitates the prediction of symptom expression (Figure 3). The input of temperatures and precipitation allows the producer to determine the likelihood that visual inspection will detect BRR symptoms in foliage or tubers. This should help determine if immunological or nucleic acid testing is sufficient to detect any traces of the BRR pathogen. For example, in a year that results in poor BRR expression, it may be prudent to increase the level of testing to avoid an undetected increase in BRR infected material. A beta version of the program is available to PGA producers.

**Figure 3.** An executable program developed from BRR data facilitating the prediction of symptom expression and vigilance in testing required to ensure absence. A beta version of the program is available to PGA members.




Sensitive pathogen-specific polymerase chain reaction diagnostics have been developed that are capable of quickly detecting trace levels of nucleic acid from the blackleg and bacterial ring rot pathogens. The procedure works on extremely small samples of only a few milligrams, may be used to examine any sample including soil, and results can be available within only a few hours. The procedures are quantitative facilitating the estimation of pathogen levels in seed or soils before planting and are capable of differentiating between strains with different characteristics such as aggressiveness and symptom expression. Initial results show little variation in the hypervariable intergenic regions of the ribosomal DNA from the pathogen causing bacterial ring rot but a surprisingly large level of variation has been observed in the blackleg samples (Figure 4). This may explain why the blackleg in some areas has been relatively difficult to eradicate and suggests there may need to be different strain specific treatments. However, no samples of *Erwinia chrysanthemi* causing stem wet rot in rapidly expanding areas of Europe or *Erwinia braziliensis*, an aggressive species found in South America.

**Figure 4.** Alignment of several rDNA intergenic sequences from *Erwinia* species isolates. The first 3 sequences represent *E. braziliensis*, the following 4 sequences are from *E. carotovora*, and the last 2 sequences are from *E. chrysanthemi*. Each of the four nucleotides is indicated by a different colour. At least three types of blackleg pathogen have been identified by the nucleotide sequence.



Probiotics

Several virulent soil probiotics that aggressively attack blackleg and bacterial ring rot pathogens have been isolated and are being characterized for application as a seed treatment and in furrow amendment that prevents blackleg and ring rot (Figure 5). Greenhouse and field trials have been established for the evaluation of disease symptom expression in potato varieties, characterization of the diagnostics, and determination of the most effective application parameters for the prevention measures. Producers are encouraged to continue submitting diseased tissues and soil samples for confidential evaluation and thereby assist in increasing the number of isolates and strains available for characterizing the diagnostics and prevention strategies. The Pesticide Management Regulatory Agency has approved the application of the probiotics by the seed industry for evaluation and producers are encouraged to contact us to arrange shipment and collaborative testing.

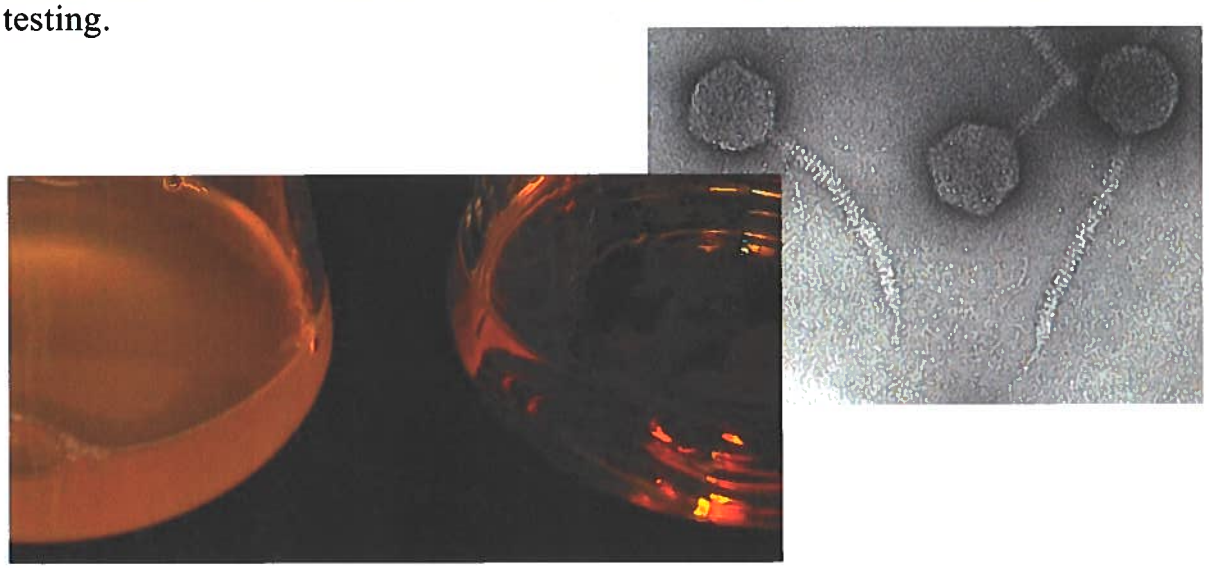


**Figure 5.** An overnight culture of the blackleg pathogen *Pectobacterium atroseptica* (Syn. *Erwinia carotovora atroseptica* ) (left) treated with an aggressive virulent phage isolated from Canadian soil (right). Greenhouse trials have confirmed the efficacy of the proactive cultures and Pest Management Regulatory Agency has approved commercial seed trials. Initial results also suggest the phage will be effective against biofilms that have made ring rot and blackleg difficult to prevent.

**Technology Transfer**

Disease control information and strategies have been communicated to producers and industry through presentations at the PGA Annual Meeting in Kananaskis, research tours, and in publications. The bacterial ring rot field trial at the AAFC Stavely Substation is the only such site in Canada and was re-established to continue 30 years of screening. Advanced lines planted in field trials by industry and AAFC to evaluate symptom expression for blackleg and bacterial ring rot. Harvested tubers were evaluated for disease in storage and effectiveness of control. Reports that summarize diagnostic capabilities, control strategies, and symptom expression are being collected, analyzed, and distributed to industry. Licenses will be obtained for commercializable products and the diagnostics transferred to service labs in western Canada. Patent applications will be prepared as warranted to capture commercializable products and technologies. Progress reports will be prepared annually and a final report submitted at the conclusion of the study.

L. Kawchuk. 2007. Evolution and Eradication of Blackleg. Invited Symposium Presentation. PGA Annual Meeting. Kananaskis, AB.

L. Kawchuk, R. Howard, and B. Bizimungu. 2007. Evaluation of incidence and prevention of blackleg and bacterial ring rot. PGA Annual Meeting Poster. Kananaskis, AB.

L. Kawchuk, R. Howard, B. Bizimungu, and S. H. De Boer. 2007. Characterization of the blackleg pathogen in potato. Plant Pathology Society of Alberta Annual Meeting Presentation. Lethbridge, AB.

L. Kawchuk. 2007. Potato Molecular Improvement Tools. Western Potato Council, Vancouver, BC.

Bizimungu, B., Lynch, D.R., Kawchuk, L.M., Chen, Q., Konschuh, M., Holley, J., Fujimoto, D.K., Driedger, D., Wolfe, H., Dunbar, L., Waterer, D., Bains, P., Wahab, J. and McAllister, P. 2007. Northstar: A high yielding white cold-storage chipping potato cultivar with attractive, oval tubers resistant to late blight. American Journal of Potato Research 84: 457-465.

Kawchuk, L.M. and Kalischuk, M.L. 2007. Plant disease resistance genes. In *"Recent Research Developments in Plant Genetics"*. Ed. S.G. Pandalai. Research Signpost. (in press)

## Economical and Environmental Benefits

Apparent increases in blackleg and bacterial ring rot in western Canada are associated with reduced yields and quality or decertification that adversely impacts producers and processors. These pathogens, especially bacterial ring rot, also adversely impact trade and are sometimes used as a non-tariff trade barrier. Acquisition and characterization of endogenous pathogen populations will facilitate the development of diagnostic procedures to assist in reliable early detection and to reduce disease occurrence. Results will advance our understanding of host-pathogen interactions and identify effective disease control strategies that help reduce the occurrence of blackleg and bacterial ring rot such as cost-effective phage biocontrol. Control measures for blackleg and bacterial ring rot in western Canada will improve the sustainability and competitiveness of the potato industry in Alberta.

## Acknowledgements

We gratefully acknowledge the support of the Potato Growers of Alberta, Maple Leaf Potatoes, the Canadian Food Inspection Agency, and the Agriculture and Agri-Food Canada Matching Investment Initiative. Industry is invited to test the probiotic seed treatment and continue submitting samples for confidential evaluation to assist with the development of diagnostics and prevention measures.

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**Part 1B – Corporation/Cooperative/Partnership**

**ADVANCE PAYMENTS PROGRAM (APP)**
**APPLICATION & REPAYMENT AGREEMENT – CORPORATION/COOPERATIVE/PARTNERSHIP INFORMATION**
**PROTECTED "A" ONCE COMPLETED**

## 1.1 DOCUMENTATION

✓ Administrators must request documentation from the Shareholders, Members or Partners.

☒ X Identity verified (Photo identification required, i.e. driver's license. Medicare card cannot be accepted.)

Type of documentation provided: Drivers License's

☐ Certificate of Incorporation / Proof of Partnership

## 1.2 BASIC INFORMATION

✓ Identify the legal name of the Corporation/Cooperative/Partnership applying for the advance and indicate the type.
✓ List all Shareholders, Members or Partners of the Corporation/Cooperative/Partnership. Attach a separate sheet if required.
✓ If the ownership structure has changed from the previous application please attach the new Incorporation/Cooperative/Partnership document that reflects the correct ownership structure.

| Legal Name of Business: | APP ID of Corporation/Cooperative/Partnership: |
|---|---|
| Chin Coulee Farms Ltd. | 79521 |

Indicate type of business: ☐ Corporation ☐ Cooperative ☐ Partnership ☐ Other:

| APP ID | First Name | Last Name | Address | Phone Number | Date of Birth (yyyy/mm/dd) | % Interest in Operation |
|---|---|---|---|---|---|---|
| ~~4609632588~~ | ~~Anne~~John | ~~Vossebelt~~Cailliau | ~~Box 1314, Coaldale, AB T1M 1N1~~Box 1859, Enchant AB. T0K 0V0 | ~~403-345-2156~~403-739-3785 | ~~1952-11-08~~1948-07-04 | ~~50.1 %~~50 % |
| ~~4609632587~~ | JohnGiselle | ~~Vossebelt~~Cailliau | ~~Box 1314, Coaldale, AB T1M 1N1~~Box 1859, Enchant AB. T0K 0V0 | ~~403-345-2156~~403-739-3785 | ~~1949-03-29~~1955-02-08 | ~~49.9 %~~50 % |
| | | | | | | % |
| | | | | | | % |

**Mailing Address of Corporation/Cooperative/Partnership:**

| Street Address | City/Town | Province | Postal Code |
|---|---|---|---|
| ~~4910 40 St As Above~~ | Taber | AB | T1G 1C8 |

| Business Phone # (Ext.) | Business Fax # |
|---|---|
| | |

## 1.3 DECLARATION OF APP ADVANCE RECEIVED FROM OTHER PRODUCER ORGANIZATIONS

✓ Outstanding advances previously issued or attributed to any Shareholder, Member or Partner will affect the amount of the advance the applicant may be eligible to receive.
✓ List all advances issued by other organizations for this production period and previous production periods to each Shareholder, Member or Partner of the business, including advances attributed by percentage of ownership in any Corporation/Cooperative/Partnership.
✓ Attach a separate sheet if required.

| Name of Shareholder, Member or Partner | Name of Producer Organization from which an advance has been received | Agricultural Product for which an advance has been received | Production Period | Amount of Advance Received |
|---|---|---|---|---|
| | | | 20 | $ |
| | | | 20 | $ |
| | | | 20 | $ |
| | | | 20 | $ |

## 1.4 LENDER INFORMATION

**Name of Lending Institution**
First Choice Credit Union~~Bank of Nova Scotia~~

| Street Address | City/Town | Province | Postal Code |
|---|---|---|---|
| 45 Fairmont Blvd 1921 20 Ave. | ~~Coaldale~~Lethbridge | AB | T1M-1N2T1K 1T1 |

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**Part 1B – Corporation/Cooperative/Partnership**

**ADVANCE PAYMENTS PROGRAM (APP)**
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| | | | |
|---|---|---|---|
| | | | |

| Phone # (Ext.) | Business Fax # |
|---|---|
| 03-320-460045-0130 | |

## .5 RELATED PRODUCERS

- Producers are related if they do not deal with each other at arm's length.
- In the absence of proof to the contrary, producers are presumed not to deal with each other at arm's length if they are related as follows:
  - ✓ Individuals who are cohabitating or are connected by blood relationship, marriage, common law partnership or adoption;
  - ✓ A corporation and a person who holds shares in the corporation, is a member of a group of persons that holds shares in the corporation, or is related to a person who holds shares in the corporation or to the member of the group;
  - ✓ Any two corporations if one person or group of persons holds shares in both corporations, a person who holds shares in one of the corporations is related to a person who holds shares in the other corporation, a person who holds shares in one of the corporations is related to a member of a group of persons that holds shares in the other corporation, or if they are both related to a third corporation;
  - ✓ Members of the same group of persons; or
  - ✓ Persons related to members of the same group of persons.
- Relatedness affects the applicant's eligibility to receive an advance, as well as the amount of an advance.
- If you answer "yes" to question 1 below, complete section 1.5.1 which is a declaration of relatedness.
- If you answer "yes" to question 2, you may not be eligible to receive an APP advance, unless you are able to rebut the presumption of relatedness.
- If you answer "no" to questions 1 and 2, you are not required to complete section 1.5.1.

| | | |
|---|---|---|
| . Has a related producer a) applied for an APP advance in this production period or b) have an outstanding APP advance from a previous production period? | YES | NO |
| . Is any related producer ineligible as a result of a default under APP, SCAP or ESCAP? | YES | NO |

## 1.5.1 RELATED PRODUCER DECLARATION

- ✓ List all related producers who received an advance for this or previous production periods, including advances issued by other APP Administrators.
- ✓ Attach a separate sheet if required.

| Name of the related producer that received an advance | APP ID | Name of Administrator which issued the advance | Production period (yyyy) |
|---|---|---|---|
| | | | |
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**Part 1B – Corporation/Cooperative/Partnership**

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- List all related producers who received an advance for this or previous production periods, including advances issued by other APP Administrators.
- Attach a separate sheet if required.

| Name of the related producer that received an advance | APP ID | Name of Administrator which issued the advance | Production period (yyyy) |
|---|---|---|---|
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## 1.6 GUARANTEE DECLARATION

**PERSONAL GUARANTEE (for Corporation with sole shareholder)**

I, being the sole shareholder of the Corporation stated in Section 1.2 of this Application for an Advance in consideration of an advance being made to it by the Administrator, for the amount stated in Part 2 of this Application for an Advance, for the 2013-2014APP production period and the Minister of Agriculture and Agri-Food Canada guaranteeing the repayment of such advance and interest thereon, do hereby agree to be personally liable to the Administrator or the Minister of Agriculture and Agri-Food Canada for any amount owing by the Corporation, under the APP.

***By signing this document, you understand and agree that action may be taken against you personally to be liable under Section 5.0 of the Terms and Conditions of the Repayment Agreement and to repay the full amount of any defaulted advance.***

IN WITNESS WHEREOF I hereunto set my hand and seal

Dated at _____ _____

*Location* Date (YYYY-MM-DD)

_____ _____
Print name of shareholder clearly Signature of shareholder

_____ _____
Print name of witness clearly (Must not be a relative) Signature of witness

**JOINT AND SEVERAL GUARANTEE (for Cooperative, Partnership or Corporation with multiple shareholders)**

We, being Shareholders, Members or Partners, as the case may be, of the Corporation, Cooperative or Partnership as stated in Section 1.2 of this Application for an Advance, in consideration of an advance being made to the Corporation, Cooperative or Partnership, as the case may be, by the Administrator for the amount stated in Part 2 of this Application for an Advance, for the 2013-2014 APP production period and the Minister of Agriculture and Agri-Food Canada guaranteeing the repayment of such advance and interest thereon, do hereby agree to be jointly and severally liable to the Administrator, or the Minister of Agriculture and Agri-Food Canada, for any amount owing by the Corporation, Cooperative or Partnership, as the case may be, pursuant to the APP.

***By signing this document, you understand and agree that action may be taken against you personally to be liable under Section 5.0 of the Terms and Conditions of the Repayment Agreement to repay the full amount of any defaulted advance.***

IN WITNESS WHEREOF I hereunto set my hand and seal

Dated at _____AB_____ – 2013/05/132014/01/20

*Location* Date (YYYY-MM-DD)

John VossebeltJohn Cailliau
_____ _____
Print name of shareholder, member or partner clearly Signature of shareholder, member or partner

_____ _____
Print name of witness clearly (Must not be a relative) Signature of witness

Giselle CailliauAnne Vossebelt
_____ _____
Print name of shareholder, member or partner clearly Signature of shareholder, member or partner

_____ _____
Print name of witness clearly (Must not be a relative) Signature of witness

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**ADVANCE PAYMENTS PROGRAM (APP)**
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| Print name of shareholder, member or partner clearly | Signature of shareholder, member or partner |
|---|---|
| Print name of witness clearly (Must not be a relative) | Signature of witness |