Early Dying and Oomycete Analysis and Control

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 early dying and oomycete pathogens. The procedures work 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. Application of the developed procedures for the detection of nematodes has proven extremely useful. Over 200 diseased tissue and soil samples have been examined to date. Several species of *Verticillium* were isolated from early dying samples and a number of strains of the late blight pathogen were observed compared to the lack of variation seen in the pink rot isolates. Greenhouse and field trials have facilitated evaluation of disease symptom expression in potato varieties, characterization of the diagnostics, and determination of the most effective application parameters for the control measures. Several potential probiotics have been isolated from endemic soils that are being evaluated for disease prevention. Producers are encouraged to continue submitting diseased samples for confidential evaluation and thereby assist in characterizing the diagnostics and prevention strategies. Agriculture and Agri-Food Canada approved an application 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

Early dying is a common disease, caused by several different species of *Verticillium* fungi and influenced by nematodes. It occurs in most potato growing areas of the world. The incidence and severity of early dying appears to be increasing in western Canada potato producing areas. *Verticillium* species have a wide host range and are known pathogens of many crops and other plants. Disease development impedes water movement within the plant and is influenced by many abiotic and biotic factors. Early dying can cause severe yield losses and leads to internal net necrosis in many potato varieties. Soil fumigants are sometimes used to control the disease but they are expensive and essentially sterilize the soils. Several species of *Verticillium* are known to cause disease but the factors

contributing to the disease are poorly understood. Additional information on the potential transmission, detection, and control of early dying is required.

Late blight, pink rot, and leak are caused by the oomycetous fungi *Phytophthora infestans, Phytophthora erythroseptica*, and *Pythium ultimum*, respectively. They represent potentially the most devastating group of potato pathogens. The incidence of pink rot and late blight is increasing in incidence and possibly severity in western Canada but the exact cause or population dynamics remain to be determined. Late blight can decimate a crop within a few days and like pink rot, it can infect a healthy tuber. Control involves several applications of fungicide applied in a preventative manner but these pathogens have developed pesticide resistance. Our understanding of the oomycetes is still quite limited and alternatives for detection and control are required.

Diagnostics that identify pathogen/pest sources and strains and disease control strategies based on management and biocontrol, will reduce disease losses, eliminate pesticides that can adversely impact environment, and improve the competitiveness of the Alberta product.

Objectives

1) Develop diagnostic tests for reliably detecting the pathogens and pests contributing to early dying, leak, late blight and pink rot. Assays will help determine sources, vectors, and pathogen strain distribution in fields selected for potato production.

2) Characterize the pathogen/pest populations causing early dying, leak, late blight and pink rot in Alberta. Samples will be obtained from diseased tissues, soils, soil debris, and culture collections to determine virulence, aggressiveness, and other characteristics such as pesticide reaction.

3) Develop strategies for the control of early dying, leak, late blight and pink rot. This will involve a management approach based on diagnostic information, the screening of germplasm and advanced lines for resistance, storage and soil monitoring and amendments, and crop rotations.

4) Improve the competitiveness and sustainability of producers and processors by advancing our understanding of these pathogens/pests to improve yield and quality.

Methods and Materials

1) Pathogen/Pest identification, and isolation: Industry, CFIA, and collaborators will assist in collection of diseased samples and early dying, leak, late blight, and pink rot pathogen/pest identification/isolation. Additional pathogen/pest populations will be obtained from existing regional, National, and International culture collections for comparison.

2) Detection and risk levels: Sensitive pathogen/nematode polymerase chain reaction (PCR) assays will be developed/applied to detect each pathogen and pest. Universal primers designed for highly conserved rDNA sequences have proven effective in reliable identifications of pathogens and other organisms. Testing will examine various sources of the pathogens and nematodes including field soil, alternative hosts, and seed to determine inoculum loads and risk.

3) Strain characterization: AAFC will develop PCR assays to analyse genetic variability within each pathogen/pest to identify different strains. Hypervariable intergenic spacer regions such as the rDNA ITS regions are capable of distinguishing even small variations in populations. Results will help develop multplex assays to detect several pathogens/pests and reduce test costs. PCR amplifications will be conducted under stringent conditions and amplified products cloned and sequenced. Sequence comparisons and analyses can be performed with various available software programs.

4) Disease management: Management practices and pathogen threshold values will be evaluated to determine strategies to control pathogen reservoirs and vectors and minimize disease losses. True potato seed from accessions held in germplasm repositories and advanced lines from the AAFC Potato Breeding Program will be screened with aggressive strains of early dying, late blight, and pink rot pathogens in storage, greenhouse, and/or field trials. Monitor pathogen/pest changes in soil and seed after vine removal, deep tillage, green manures, and crop rotations to reduce disease.

Results and Discussion

The project commenced in the spring of 2006. Agriculture and Agri-Food Canada approved an application to match the Potato Growers of Alberta contributions. Excellent progress has been made in both the development of diagnostics, the isolation of early dying, pink rot, late blight, and leak pathogens, and the identification of probiotics that prevent disease. Producers are encouraged to continue submitting suspect samples for confidential evaluation and thereby assist in characterizing the diagnostics and prevention strategies (Table 1).

Industry, the Canadian food Inspection Agency, and collaborators assisted with the collection of diseased samples for pathogen identification and isolation. Over 200 samples from North America were collected for development of diagnostics, characterization, and prevention strategies. Isolates were evaluated for aggressiveness and suitability in greenhouse and field trials (Table 2). Several of the more aggressive isolates were selected for screening advanced lines and varieties for symptom expression and eventually effectiveness of diagnostic and control measures. Additional pathogen strains will be obtained from existing regional, National, and International culture collections for comparison.

Several species of *Verticillium* were recovered from early dying samples. This appears to include species previously not known to infect potato. Each species has intrinsic properties that may influence the damage inflicted on the crop. For example, *Verticillium dahliae* produces a tough thick walled resting stage microsclerotia that can potentially overwinter in soils. Further analysis will determine the prevalence of each species and characteristics that may assist in controlling each pathogen. Comparison of rotations by Dr. F. Larney at the AAFC Vauxhall Substation has resulted in the prevention of early dying (Figure 1). Initial soil analysis has shown little variation in the pathogen levels but changes in the beneficial microflora including the increased presence of a probiotic *Plectosphaerella cucumerina* that is known to attack nematodes. Nematodes are an important component of the early dying complex.

Figure 1. Pictures from adjacent plots in 2007 of a 5 yr sustainable rotation (right) versus a 3 year conventional rotation (left). Although the soil samples showed only a slight reduction in the level of the verticillium wilt pathogen in the sustainable rotation, the microflora has changed with an increase in beneficial microorganisms as determined with the developed diagnostics.

No *Phytophthora infestans* was detected in any of the samples obtained from Alberta in 2006/07 and 2007/08. A number of strains were observed amongst the *Phytophthora infestans* from other provinces, providing the ability to track strain distribution and spread (Table 1). Surprisingly pink rot appears to be increasing and the *Phytophthora erythroseptica* from all provinces showed relatively little variation amongst strains and suggests a relatively uniform pathogen population. Analysis of association of observed differences with pathogen traits such as pesticide resistance has not been observed to date. Analysis of several leak isolates has also shown limited strain variation (Figure 3).

Table 1. Characterization of Canadian *Phytophthora infestans* isolates.

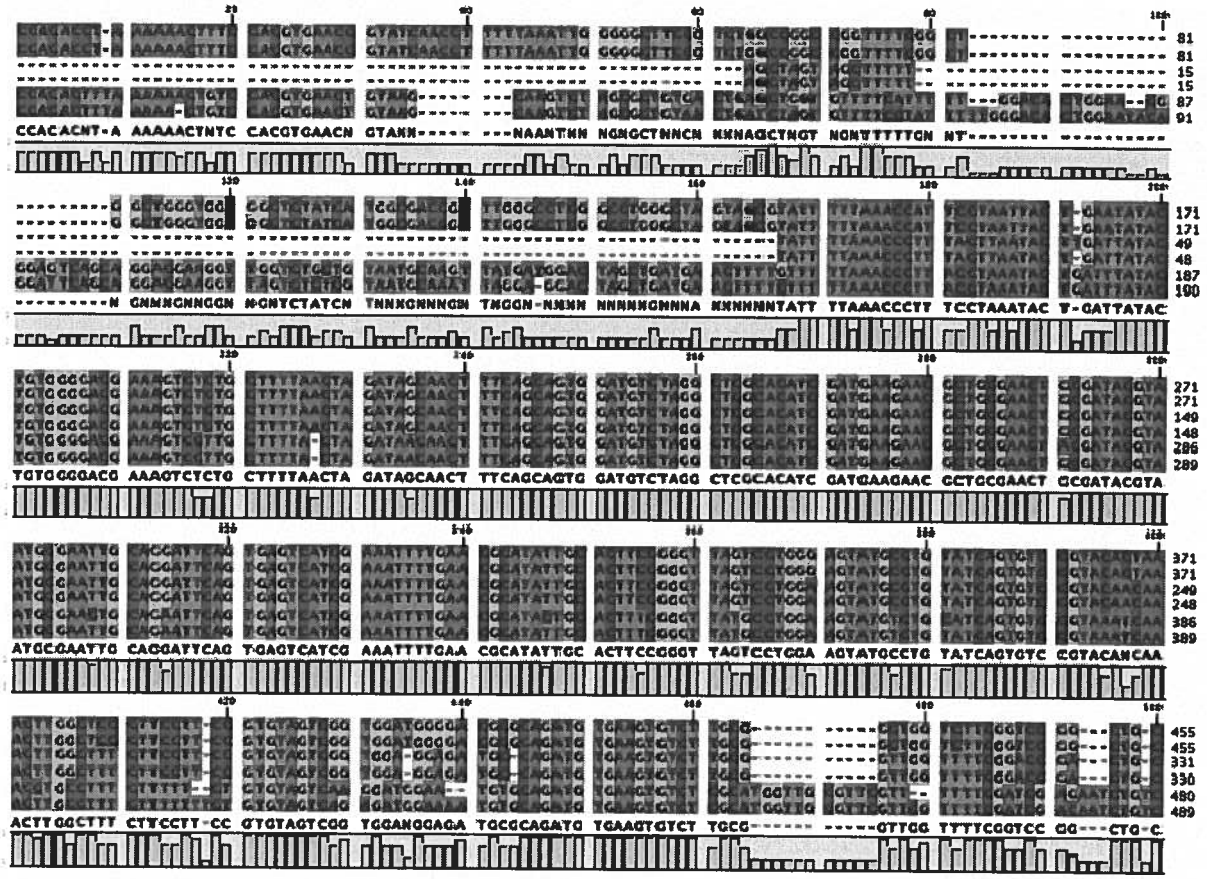
Mating Type	Metalaxyl Response	Genotype	Pathotype
A1	Intermediate	US11	2,4,6,9/1,3,5,7,8,10,11
A1	Resistant	US11	2,6,9,10/1,3,4,5,7,8,11
A1	Sensitive	US6	2,4,6,9/1,3,5,7,8,10,11
A1	Sensitive	US6	2,9/1,3,4,5,6,7,8,10,11
A1	Intermediate	US6	2,4,6,9/1,3,5,7,8,10,11
A1	Resistant	US11	2,9,10/1,3,4,5,6,7,8,11

Allozyme analysis of the *P. infestans* isolates was performed on pre-treated cellulose acetate gel stained for the *Glucose phosphate isomerase* (*Gpi*) locus. Results from the CAE of *Gpi* closely correspond with the expected mating type and metalaxyl response for US-6 and US-11 genotypes. Further analysis revealed five different pathotype races for the six isolates (Table 1). Mating type determination of isolates was made with isolates of known A1 or A2 genotypes and the presence of oospores. Only A1 mating types were observed. Metalaxyl response was determined by comparing the growth rate of isolates on modified CRA plates (mCRA) made with 1g glucose/l augmented at 100 ug/ml metalaxyl M (active isomer of metalaxyl) as found in Ridomil Gold (49.5% w/w) liquid formulation, and the growth rate on mCRA plates with no metalaxyl present. Percent growth was calculated and the response rated as Metalaxyl Sensitive: <10%, Metalaxyl Intermediate: 10-60%, and Metalaxyl Resistant: >60%. Metalaxyl response revealed that two of the three US-6 isolates were sensitive and one was intermediate (Table 1). Of the three US-11 isolates two were resistant and one was intermediate to metalaxyl. Race profiles of the *P. infestans* isolates were determined by screening for pathogenicity on eleven single R-gene differentials of *Solanum demissum.*

Table 2. Late blight foliage 2007 disease in potato varieties and advanced lines. Assays used an isoloated aggressive and highly virulent US8 *Phytophthora infestans* genotype.

Cultivar or Line	Reaction	Rating
CV96022-3	Moderate	**2.8**
CV96053-4	Moderate Resistant	**2.0**
CV97006-1	Moderate Susceptible	**3.8**
CV97050-3	Moderate	**2.6**
CV97065-1	Moderate	**2.8**
CV97112-5	Moderate	**2.2**
CV97123-1	Moderate	**2.2**
CV99279-1	Moderate	**2.6**
V0319-1	Moderate	**2.4**
V0379-2	Susceptible	**4.0**
V0950-3	Moderate	**2.2**
V1102-1	Moderate Resistant	**1.6**
WV3667-1	Moderate	**2.4**
Stirling CP487	Resistant	**0.2**
Shepody CP633	Susceptible	**4.4**
Russet Burbank	Moderate Susceptible	**3.0**

Figure 2. Alignment of several rDNA intergenic sequences from isolates of the oomycete pathogens: first 2 sequences are *Phytophthora erythroseptica*, middle 2 sequences are *Phytophthora infestans,* and the last 2 sequences are *Pythium ultimum.* Each of the four nucleotides is indicated by a different colour. Several strains of the pathogen are evident and these differences should facilitate tracking and avoidance.



Technology Transfer

Disease control information and strategies have been communicated to producers and industry through presentations and publications. Advanced lines will be planted in field trials at various locations by industry and AAFC to evaluate agronomic performance and disease resistance. Harvested tubers will be evaluated for disease in storage. Reports that summarize diagnostic capabilities, control strategies, and disease/pest resistance will be collected, analyzed, and distributed to the industry. Licenses will be obtained for the various products that are commercializable and 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.

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Economical and Environmental Benefits

Apparent increases in early dying, leak, late blight and pink rot in western Canada are associated with reduced yields and quality that adversely impact producers and processors. These diseases also often compromise healthy tubers, predisposing potatoes to secondary diseases such as fusarium dry rot. Acquisition and characterization of endogenous pathogen/pest populations will facilitate the development/application of cost-effective multiplex diagnostic procedures to assist in early reliable detection of the pathogen/pests in soils, seed, and other sources to avoid disease. The identified differences allow the pathogens to be

tracked and management decisions may be made in regards to levels of the pathogen in advance of planting or application of pesticides. Results have advanced our understanding of host-pathogen interactions and identify effective alternative disease control strategies that help reduce pesticide applications thereby addressing growing health and environmental concerns. Better control measures for early dying, leak, late blight and pink rot in western Canada through integrated pest management and probiotics will improve the sustainability and competitiveness of the potato industry in Alberta.

Acknowledgements

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