Early Dying and Oomycete Analysis and Control

Potato Growers of Alberta
Progress Report 2006/07

L. Kawchuk, R. Howard, and H. Platt

Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB

Alberta Agriculture, Food and Rural Development, CDC South, Brooks, AB

Research Centre, Agriculture and Agri-Food Canada, Charlottetown, PEI

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. Initial results show different strains and species variation in the pathogens from diseased samples. Several species of *Verticillium* were isolated from early dying samples that may complicate control and a surprisingly large 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 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 control measures. Producers are encouraged to submit suspect samples for confidential evaluation and thereby assist in characterizing the diagnostics and prevention strategies. Agriculture and Agri-Food Canada has approved an application to match the Potato Growers of Alberta project contributions.

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 mangement 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 has approved an application to match the Potato Growers of Alberta contributions. Excellent progress has been made in both the development of diagnostics and the

isolation of aggressive virulent isolates of early dying, pink rot, late blight, and leak. Producers are encouraged to continue submitting suspect samples for confidential evaluation and thereby assist in characterizing the diagnostics and prevention strategies.

Industry, the Canadian food Inspection Agency, and collaborators assisted with the collection of diseased samples for pathogen identification and isolation. Approximately 100 samples 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. 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.

Surprisingly, a large number of strains were observed amongst the *Phytophthora infestans*, providing the ability to track strain distribution and spread. Interestingly *Phytophthora erythroseptica* 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 are underway. Analysis of several leak isolates is also in progress.

Figure 1. Screening of potato varieties and advanced lines with isolated verticillium wilt pathogen assists in developing resistance and determining the strains of pathogen present. The two plants on the left are resistant to a local aggressive virulent isolate of a *Verticillium* spp. and do not show symptoms of early dying whereas the susceptible plants on the right are showing severe wilt symptoms.

Table 1. Late blight tuber disease in potato varieties and advanced lines (N=10). Assays performed with an aggressive and highly virulent US8 *Phytophthora infestans* genotype (LRC05.2).

Tuber Variety	Average	Rating	Standard Error
Shepody	3.06	S	0.29
Russet Burbank	2.30	M	0.20
CV97192-1	**1.40**	**R**	**0.29**
CV95002-1	2.00	M	0.35
CV97065-1	2.60	M	0.32
CV97112-5	**1.70**	**R**	**0.40**
CV92028-1	2.10	M	0.33
V1102-1	3.10	S	0.33
FV12469-1	3.00	S	0.24
FV12486-2	**1.60**	**R**	**0.32**
CV97105-2	2.40	M	0.45
CV96047-1	3.00	S	0.28
CV97006-1	2.90	M	0.22

Disease Ratings: 0 = 0%; 1<10%; 2<25%, 3<50%; 4<75%; 5<100%

Figure 2. Tubers inoculated with the late blight pathogen produce rapid breakdown of the tuber allowing secondary pathogens such as those causing fusarium dry rot. The diagnostics will facilitate the monitoring for the presence of the pathogen in advance of the disease appearing in the field and may assist in reducing the number of proactive pesticide applications required.

Figure 3. Tubers incoculated with the leak pathogen quickly develop distinctive tuber symptoms. Sources of the pathogen and methods to reduce disease are being examined with the help of the developed diagnostics. The diagnostics should provide a valuable tool for checking field samples for levels of the pathogen prior to planting.

Figure 4. Alignment of several rDNA intergenic sequences from early dying verticillium wilt pathogen isolates. Each of the four nucleotides is indicated by a different colour and several different species appear to be involved in the disease.

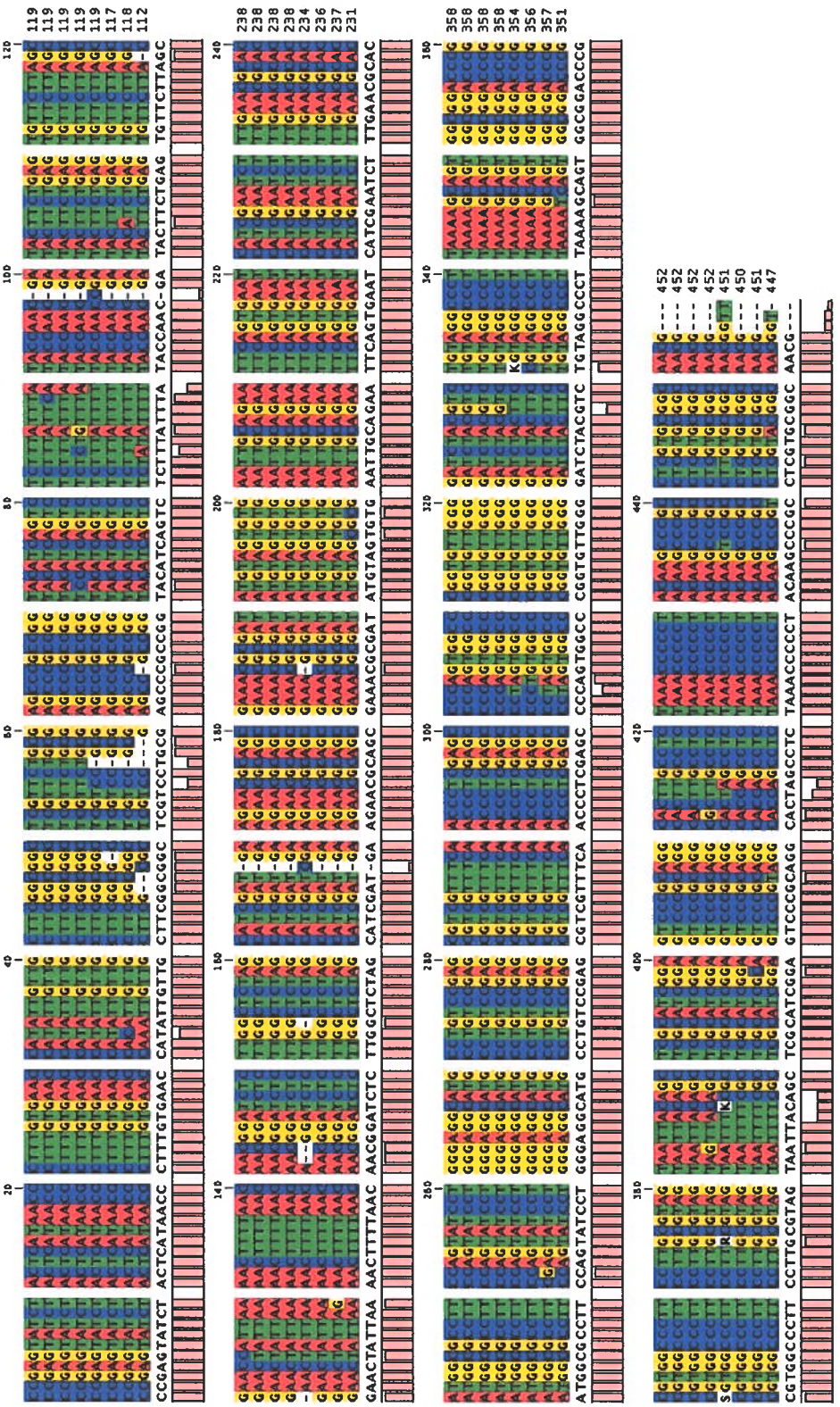


Figure 5. Alignment of several rDNA intergenic sequences from isolates of the late blight pathogen. Each of the four nucleotides is indicated by a different colour. Several strains of the pathogen are evident and these differences should facilitate tracking and avoidance.

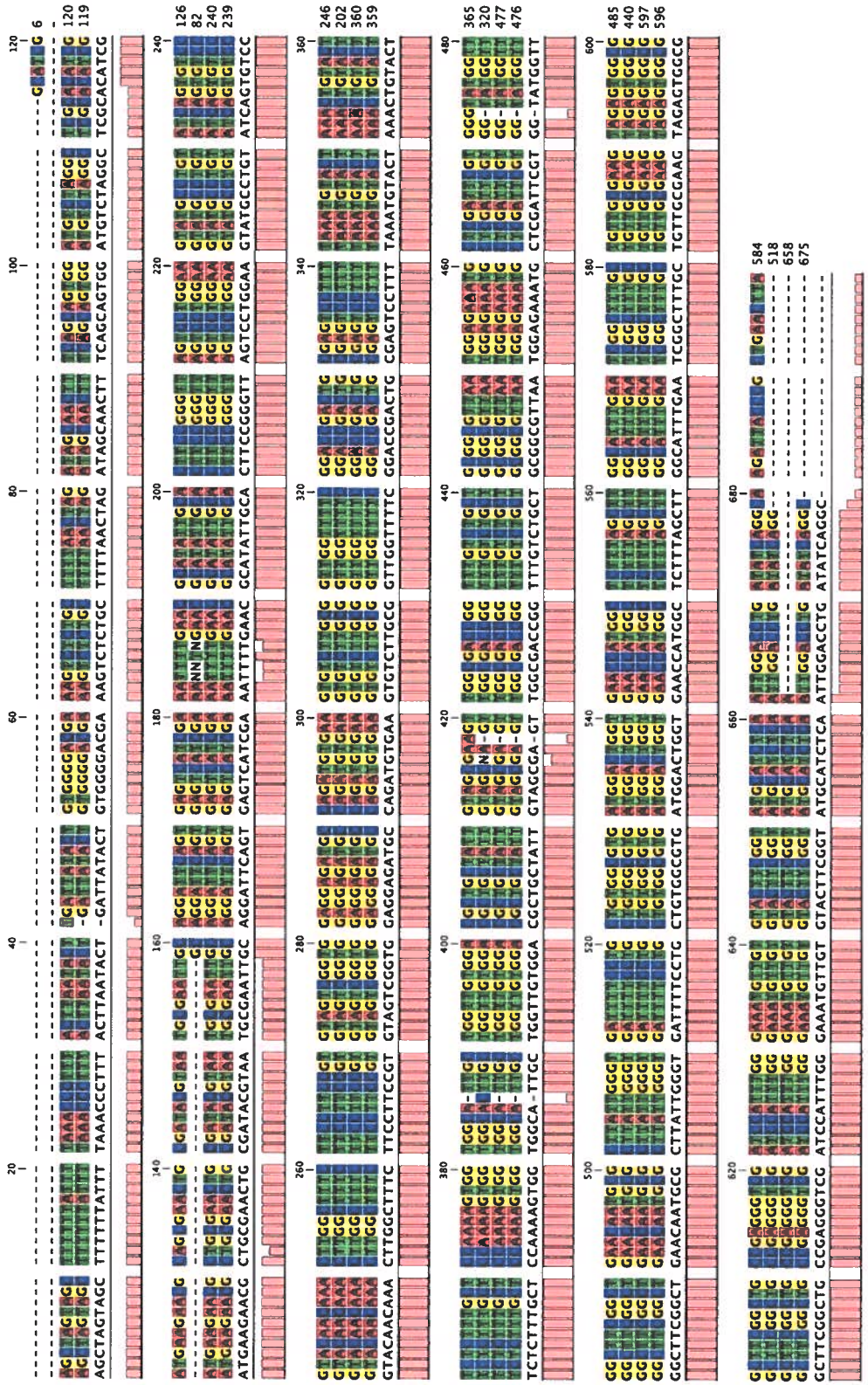
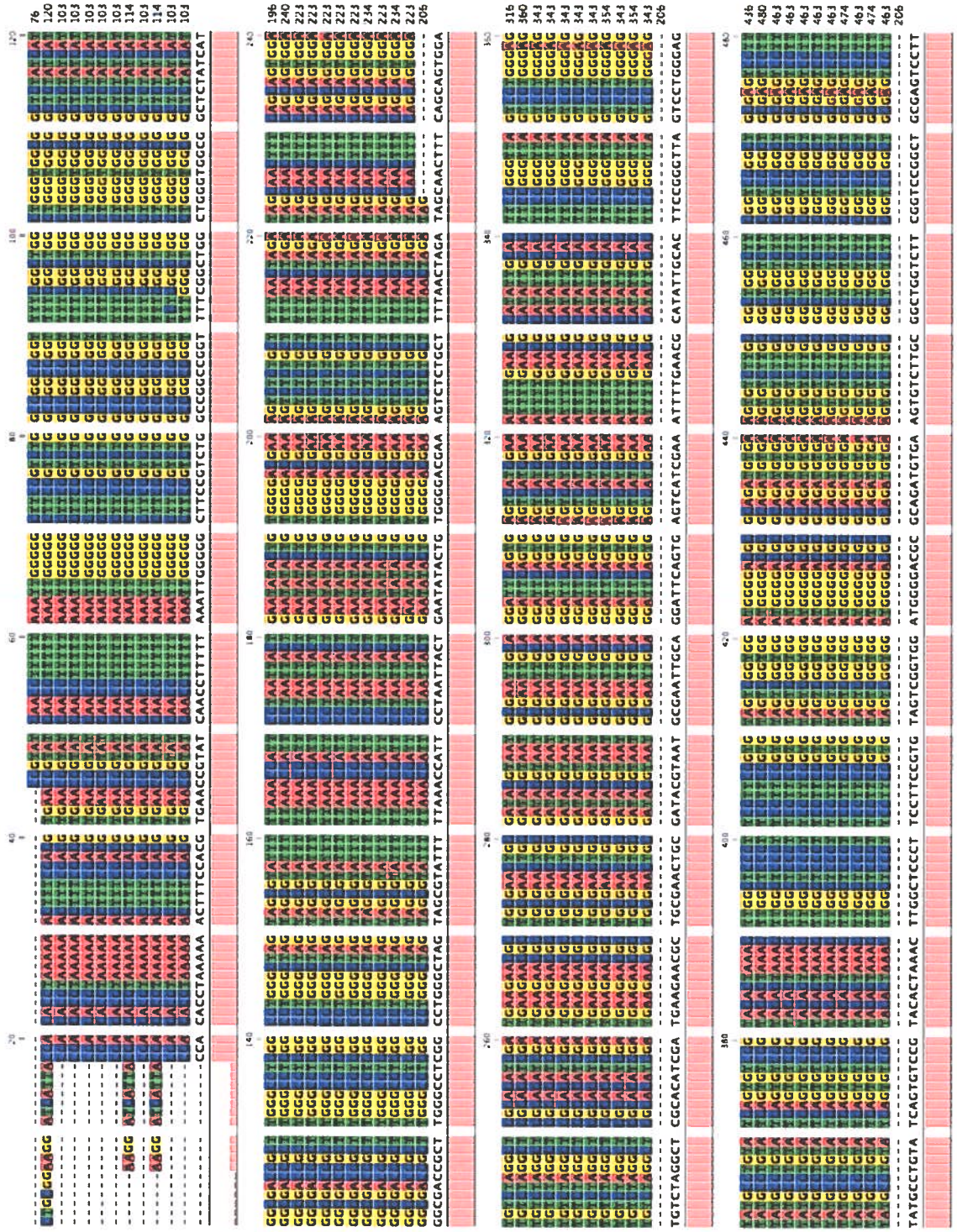


Figure 6. Alignment of several rDNA intergenic sequences from isolates of the pink rot pathogen. Each of the four nucleotides is indicated by a different colour. Few strains of the pathogen are evident suggesting that control may be relatively simple and involve restriction of movement of disease material.



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.

L. Kawchuk. 2006. Potato Molecular Improvement Tools. Bulletin. Lethbridge, AB.

L. Kawchuk. 2006. Poatato Disease Prevention. Maple Leaf Potatoes Invited Presentation. Lethbridge, AB.

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 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, and the Agriculture and Agri-Food Canada Matching Investment Initiative. Industry is invited to continue submitting samples for confidential evaluation to assist with the development of diagnostics and prevention measures.