

Rapid *Aphanomyces* Root Rot (PCR) Test For Alfalfa

Purpose:

Aphanomyces root rot is caused by the alfalfa pathogen *Aphanomyces euteiches* and similar to *Phytophthora* root rot (*Phytophthora medicaginis*) it is considered a major disease of alfalfa seedlings and adult plants. The incidence of *Aphanomyces* in the mid-west and northeastern United States has increased in recent years and has recently been confirmed in Ontario. These observations are of great concern since variety genetic resistance or tolerance has been the cornerstone of disease management in alfalfa production systems in Ontario. Race 1 resistant varieties and the more recent development of race 2 resistant varieties are available and a rapid diagnostic test would be beneficial to OMAFRA and the Ontario Forage Council monitoring and education efforts for *Aphanomyces* and other alfalfa diseases in Ontario.

A greenhouse differential assay has been developed for *Aphanomyces* and modified for Ontario by OMAFRA and the Ontario Forage Council but results can be influenced by external factors such as greenhouse conditions, etc. The purpose of this project was for OMAFRA and the Ontario Forage Council to link with the substantial resources of the Agriculture and Agri-Food Canada biodiversity-bioinformatics cluster in Ottawa led by Dr. Andre Levesque to compare published PCR and novel 454 molecular sequencing technologies for *Aphanomyces euteiches* and determine applicability of these methods into commercially available plant and soil diagnostic tests for producers.

Methods:

OMAFRA Ridgetown collected soil and plants from alfalfa fields across the province. Soil and alfalfa root samples were then sent to and processed at AAFC Ottawa (Dr. Andre Levesque) in order to collect the fungal DNA for molecular sequencing "prosequencing" in order to characterize all oomycetes present, i.e. *Aphanomyces* species and all closely related species. We used these fully characterized soil samples to compare the efficacy and validate the currently available PCR tests for *Aphanomyces euteiches*.

Results:

Before determining or implementing a robust diagnostic test it is imperative that we better understand the baseline level of *Aphanomyces* and closely related species that could interfere with the tests in Ontario thereby reducing confusion and false positives. We were able to demonstrate that published molecular tests cross reacted with other closely related fungi but the "novel 454" next generation sequencing had no such difficulty. By using these new technologies we were able to detect *Aphanomyces* from various root and soil samples collected from Ontario without false positives.

Over 1.4 million of oomycete sequences of an average of 400 base pairs were obtained from the samples we processed. We originally planned to do 1M sequences. Close to 350 *Aphanomyces euteiche* sequences were found from all these oomycete sequences, showing the preponderance of genera such as *Pythium*. In the literature there has been talk of other *Aphanomyces* races besides race 1 and 2 or the possibility of new *Aphanomyces* species being involved in varietal resistance breakdown. One of the

unexpected outcomes of this project was the detection of most likely a new species of *Aphanomyces* in Ontario. This is an exciting discovery and we will research this new species further to determine its risk to alfalfa production and determine if present elsewhere in North America.

The significant amount of efforts undertaken in this project in terms of molecular protocol development and sample processing has not only provided an excellent base (baseline) for enhanced forage pathogen diagnostic detection but has allowed us to validate these newest generation of powerful molecular technologies for forage production. In addition this will allow for the incorporation of forages into other projects such as future DNA barcoding projects further enhancing the industry in the future. We focused on *Aphanomyces* for this project but this is the most exhaustive survey on oomycetes in alfalfa ever done. The AAFC overall database has 55M sequences so far from various projects but this is the only commodity based project done so far.

Summary:

We showed for the very first time that next generation 454 sequencing can be used not only to detect *A. euteiches* but also to assess the diversity of any other *Aphanomyces* species closely related to *A. euteiches*. The usefulness of this technology was used to identify a new *Aphanomyces* species in one soil sample, something that the traditional PCR detection could not have done and would have taken several intensive months of work to do by a taxonomy specialist.

Differentiating races directly by molecular assays will require much more fundamental work on genomics of host pathogen molecular interactions but it appears that by monitoring the pathogen alone one can at least see when resistance breaks down. With a higher sensitivity, maybe this would be detected before higher disease levels appear in a season. This information would also be critically important to forage breeders, thereby allowing them to anticipate potential disease resistance in the field and introduce new varieties which will limit potential losses as well as maintain a sustainable and competitive forage industry in the province.

Next Steps:

We have had interest from diagnostic service laboratories in the province and would pursue the transfer of these tests to them for deliver to the Ontario forage industry thereby helping maintain a sustainable and competitive forage industry into the future. Until this forage project, the next generation sequencing detection efforts of AAFC biodiversity-bioinformatics group in Ottawa has had primarily focused on spores collected in air and rain samples and some soil at the Central Experimental Farm. Root and soil from commercial forage production fields is the first commodity based project of this kind and plans are in development to use these new technologies in other crops in the future such as cereals.

Acknowledgements:

We would like to thank the Ontario Forage Council who obtained funding through the Farm Innovation Program a component of Growing Forward 1 and administrated by the Agricultural Adaptation Council in Guelph for this project and the companion alfalfa disease survey project. It was a delight and we were fortunate to work with a world leader such as Dr. Andre Levesque at AAFC Ottawa and his lab that put up with our

Crop Advances: Field Crop Reports

many questions! We would also like to thank OMAFRA field crop extension staff and technician Cheryl Van Herk (Ridgetown) and OMAFRA students George Kotulak, Josh Johnson and Hilary Mann as well as grower cooperators who allowed sampling of their fields. Thank-You!

Project Contacts:

Albert Tenuta, OMAFRA, albert.tenuta@ontario.ca

Joel Bagg, OMAFRA, joel.bagg@ontario.ca