Grower Summary

CP 141

The molecular basis of pathogenicity of *Neonectria ditissima*

Annual/Final 2017

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Further information

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AHDB Horticulture,
AHDB
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel – 0247 669 2051

AHDB Horticulture is a Division of the Agriculture and Horticulture Development Board.

Project title:	The molecular basis of pathogenicity of *Neonectria ditissima*
Project number:	CP 141
Project leader:	Richard Harrison, NIAB EMR. Robert Jackson, Reading University.
Report:	Final report, June 2019
Previous report:	Annual report 2017
Key staff:	Antonio Gomez Cortecero, NIAB EMR
Location of project:	NIAB EMR
Industry Representative:	Tony Harding. Worldwide fruit. Acorn House, Unit 68-69, John Wilson Business Park, Harvey Drive, Chestfield, Whitstable, Kent, CT5 3QT.
Date project commenced:	Oct 2015
Date project completed:	June 2019

GROWER SUMMARY

Headline

- The genetic sequences of two isolates of *Neonectria ditissima* have been identified.

Background and expected deliverables

European canker, caused by the phytopathogenic fungus *Neonectria ditissima*, is one of the most destructive diseases of apple and pear. In the orchard, this fungus is able to infect a wide range of apple varieties causing canker and die back of young shoots, resulting in significant losses of fruiting wood. This pathogen has been reported in many apple-producing regions of the world, being especially common in the North-Western European countries. Modern varieties suffer most and in extreme cases do not survive establishment in the orchard. Canker control is difficult to achieve due to the pathogen's lifecycle which is able to infect trees all year-round through wounds, either natural, such as bud-scale scars, leaf scars, fruit scars or artificial, such as pruning wounds. Resistance breeding is underway in many global breeding programmes, but nevertheless a total resistance to canker has not yet been demonstrated in either fruit or woody tissue. There is no known race structure of the pathogen and the global level of genetic diversity of the pathogen population is unknown. Plant resistance is a promising alternative to largely ineffective cultural control but is time consuming to deploy due to the long breeding cycle in apple. Research into other host pathogen interactions shows that a dual strategy of understanding host resistance and pathogen a virulence and how the two are linked, is key to the deployment of durable resistance in the field. Nevertheless, little is known about the pathogen at the molecular level. This project is focused on dissecting components of the pathogen's genome that modulate virulence in order to understand how virulence is controlled and whether there are specific differences in host resistance response to isolates of differing virulence.

This work will provide fundamental insights into the molecular basis of pathogenicity in *Neonectria ditissima,* the causative agent of apple canker. The identification of candidates genes important in virulence in the pathogen and how these genes interacts with the host could lead to novel opportunities for control.

Summary of the project and main conclusions

Two isolates of *N. ditissima* collected in the United Kingdom were sequenced using two different long read sequencing technologies. Genome assemblies of these two isolates improved the contiguity and completeness of the genome, being an excellent resource for the study of this pathogen. Gene prediction and annotation of these genomes using RNA-Seq data allowed us to present an updated version of the predicted secretome of this pathogen.

During infection, the pathogen secretes proteins, called effectors, to modulate the host cells' response, suppressing defence and allowing colonisation. Analysis of the gene content of the genomes of the two isolates showed a full repertoire of pathogenicity genes, composed of secreted effector proteins and enzymes involved in the degradation of the plant cell walls, distributed throughout the genome. In order to identify specific genes involved in the pathogenicity, a transcriptome analysis was performed. RNA samples from artificial inoculated plants were sequenced and differentially expressed genes during the infection were identified. This analysis revealed a large number of highly expressed genes involved in the degradation of different components of the plant cell walls, mainly polygalacturonan and xylan. In addition to these genes, small secreted proteins with unknown function were also identified. These effector proteins might play a crucial role during infection, modulating the host resistant responses and allowing the colonisation of the host.

Along with the two reference genomes sequenced in this work, twenty-six isolates of *N. ditissima* from eight different countries were sequenced with the Illumina MiSeq system. Understanding the genetic variation of different population of *N. ditissima* is key for the deployment of resistance and orchard management against this pathogen. An analysis of the nucleotide diversity using four polymorphic loci was conducted at the beginning of this project. This analysis revealed slight evidence for population structure of this pathogen. To confirm these findings, analysis of the whole genome sequence of a bigger sample size was performed. This analysis revealed for the first time a clear separation between two populations within the *N. ditissima* species and evidence of hybridization.

Financial benefits

- No direct benefits have been delivered to growers from this study.

Action points for growers

- Due to the nature of this genetic based study, no action points for growers have been identified.