Grower Summary

CP 161

Understanding endophytes to Improve Tree Health

Annual 2018

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Project title:	Understanding endophytes to improve tree health.
Project number:	CP 161
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Report:	Annual report, September 2018
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Date project commenced:	1st October 2016

GROWER SUMMARY

Headline

- New findings have been made about the latent infection of *Neonectria ditissima* in apple trees

Background and expected deliverables

European apple canker (also known as Nectria canker), caused by the fungus *Neonectria ditissima*, affects both apple trees in the orchard and fruits post-harvest. In the UK, the increased planting of susceptible cultivars such as Gala and the lack of registered products for disease control, has resulted in canker becoming more serious in recent years. Evidence suggests that the pathogen can infect the tree during the propagation phase in nurseries and enter a latent phase, later developing into cankers on young trees once they are transplanted in the orchard. In some years, up to 10% of trees in newly planted orchards can be lost to canker annually. The industry is keen to focus research into:

- developing alternative control methods and
- developing a diagnostic strategy to test nursery stock material in nursery certification schemes.

The development of diagnostic methods requires good knowledge of the infection process and of disease anatomy. Currently, there are substantial gaps in our understanding of *N. ditissima* biology, which hinders the implementation of a reliable sampling strategy for diagnostics. In particular, during the asymptomatic phase of infection, we don't know whether the fungus resides in the infection site as a latent pathogen, or instead grows inside the plant's woody tissues at distance from the entry point. This peculiar lifestyle, characterised by the ability to internally colonise a plant without causing any symptoms of infection, is known as 'endophytism'.

One of the aims of this present project is to assess whether *N. ditissima* is endophytic, and to provide the basic knowledge to develop reliable sampling strategies in diagnostic technology.

In developing innovative strategies for canker control, biocontrol agents have been considered as an interesting alternative to traditional crop protection products. Most biocontrol agents are 'epiphytic', so they inhabit the surface of plants, and their efficacy as pathogen antagonists is therefore strongly dependent on environmental conditions, such as UV, humidity and temperature. However, as a part of their complex microflora (referred to as 'the plant microbiome'), plants also host fungal and bacterial endophytes. Studies have shown that some of these microorganisms interact with plant pathogens and can facilitate or antagonize them.

Therefore, together with the host genetics they can modulate disease expression, and account for part of the field resistance to Nectria canker which is observed across the commercial apple cultivars.

The second aim of this present project is to explore the endophytes associated with different apple cultivars, representative of different resistance classes, and to investigate the correlation between the cultivar endophyte profile and its resistance level.

Summary of the project and main conclusions

To pave the way for the development of reliable diagnostic tools for European apple canker in nursery stock material, different methods have been evaluated to detect *N. ditissima* in asymptomatic plant tissues. In this project, we developed a serological technique called Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of the pathogen. The ELISA is based on *N. ditissima*-specific monoclonal antibodies that can recognise the pathogen's proteins (antigens), is quick and straightforward and can be used to develop Lateral Flow Devices (LFD), which are already available as diagnostic tools for a number of fungal, bacterial and viral plant diseases. *N. ditissima* was also successfully detected in asymptomatic tissues with a method based on the detection of the pathogen's DNA called Real-Time Polymerase Chain Reaction (qPCR). Overall, he results obtained with the two techniques showed good correlation, however the ELISA appeared prone to false negatives. Further studies are required outside this current project to address the issue and develop a reliable serological technique. The DNA-based method appeared superior in specificity and sensitivity and it was chosen to carry out the disease anatomy study (see below).

To study disease anatomy during the asymptomatic phase of the infection, we have established a system to simulate a natural infection, and then assessed the localisation of the pathogen in the plant over time with the DNA-based method. Young shoots on apple trees (Royal Gala and Queen Cox) grown in an orchard at NIAB EMR were artificially inoculated via leaf scars with a spore suspension of *N. ditissima*. Plants were monitored periodically for symptom expression, and the inoculated shoots were sampled at different time points, before disease symptoms were expressed at high levels. The asymptomatic wood material was assessed for presence or absence of the pathogen by extracting the total DNA from plant tissues, and then detecting *N. ditissima*-specific DNA. The assay was performed on plant material sampled from the inoculated leaf scars and from portions of the shoots at distance from the inoculated leaf scars. Data collection is currently ongoing. However our initial results suggest that:

- Three months after the inoculation, *N. ditissima* is only found in the asymptomatic leaf scars; the pathogen was not detected in the asymptomatic internodes, at 1-1.5 cm

from the inoculation point, suggesting that no internal colonisation of the plant tissues took place in this time interval.

- After a canker lesion had developed (i.e. during the symptomatic phase), the pathogen could be found in the cankered tissue, and in the asymptomatic wood at 1-1.5 cm from the lesion, suggesting that it had spread into the apparently healthy sapwood.

A field experiment has been set-up to study the fungal and bacterial endophyte species associated to different apple cultivars representative of different disease-resistance classes. Eight different scion cultivars, grafted onto two different rootstocks, were planted in orchards at two different sites in Kent. Samples will be collected from the trees, total DNA will be extracted and DNA-sequencing technologies (Next Generation Sequencing, NGS) will be employed to identify the fungal and bacterial groups associated to plant tissues. Samples will be collected from leaf scars, which is considered the most relevant infection site for *N. ditissima* in the UK, allowing to study the endophytes which directly interact with the fungus at the entry point. We will determine the different endophyte groups and their relative amount in the different scion x rootstock combination, i.e. the cultivar-specific endophyte profiles. Moreover, we will determine whether the different profiles are correlated with the resistance level of the different cultivars assessed.

Main conclusions so far

Neonectria ditissima was detected in artificially inoculated, asymptomatic apple trees. Initial findings suggested that:

- Up to three months after the inoculation, the fungus may latently reside in the infected wound.
- Following symptom expression, spread of the pathogen in asymptomatic sapwood can occur within several centimetres from the lesion edge.

Financial benefits

Typical modern fruit wall orchards are established using around 2,800 trees per hectare. The trees cost around £5 per tree, but including wire and cane supports, they cost £7 per planting station (personal communication Nigel Jenner, Avalon Produce). With susceptible cultivars such as Gala, it is not uncommon to lose 10% of young trees to canker in the first year after establishment. This is equivalent to 280 trees costing £1,400 per hectare. These trees must be replaced which incurs additional labour costs and slows the establishment rate of the new orchard.

Developing new procedures to diagnose the presence of canker and systems for control will help to reduce the numbers of affected trees being planted and eradicate the additional expense required to replace diseased trees.

Action points for growers

The guidelines currently published on the Apple Best Practice Guide (available in the AHDB Horticulture website) provide advice to achieve effective disease management by a combination of pruning, chemical control and cultural control. Based on the literature review and on our results so far, the following additional suggestions can be given for the removal of cankers by pruning:

- Paring back of canker lesions is not recommended. Pruning should be performed instead; in fact, the fungus can be localised in every tissue between the bark and the hardwood in the lesions, therefore removal of cankered bark does not ensure removal of the infection.
- Pruning should be performed as soon as possible after a canker lesion appears. *N. ditissima* is apparently able to colonise the sapwood and move away from an actively growing lesion, without producing any symptoms.