Grower Summary

Understanding endophytes to Improve Tree Health

CP 161

Annual report 2017

Project title:	**Understanding endophytes to Improve Tree Health**
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Project leader:	Robert J. Saville, NIAB EMR
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

- Early work has paved the way for the development of novel procedures for diagnostics and control.

Background and expected deliverables

European apple canker is caused by the fungus *Neonectria ditissima* and affects apple trees in the orchard and fruits post-harvest. In the UK, the increased planting of susceptible cultivars is causing disease to become more and more important. Effective control measures are limited, due to the lack of products registered for use in apple orchards. Therefore, alternative control methods are strongly needed. Additionally, the pathogen can infect the plant during the propagation phase in nurseries and then enter a phase of latency, with the first canker lesions appearing up to three years after the trees are transplanted in the orchard. No diagnostic procedure is currently available for the early detection of this latent, asymptomatic infection.

Despite the great deal of information available on the disease epidemiology, there are substantial gaps in our knowledge of the basic biology of the pathogen which need to be addressed. An immunoassay, able to detect *N. ditissima* in infected plant material, is currently available and may be further developed for a use in diagnostic tests. However, its application requires a greater understanding of the anatomy and the temporal dynamics of the latent infection - that is where and when the fungus can be localised in plant tissues following successful infection. It is currently believed, but yet to be demonstrated, that the fungus can grow and develop asymptomatically within the plant, prior to symptom expression. This microbial lifestyle is known as endophytism.

Fungal and bacterial endophytes colonise internal plant tissues (contrarily to epiphytic microorganism, which are found on the plant surface) and cause inconspicuous and, at least transiently, asymptomatic infections. Some plant pathogens display this peculiar life-style during the early stages of infection, before switching to a pathogenic phase. Besides, several studies showed that some endophytes, acting alone or together with other endophytic species in the plant microbial ecosystem, can facilitate or antagonize plant pathogens. Therefore, they can modulate, together with the host genetics, disease expression. The study of the interactions between *N. ditissima* and the other apple tree endophytes may help to understand field resistance and provide access to untapped resources for the development of novel biocontrol strategies.

This project aims to localise the pathogen *in planta* during the asymptomatic phase of infection, with the aim of establishing an effective sampling and diagnostic procedure for the latent infection. I will also investigate the interactions between *N. ditissima* and apple tree endophytes, exploring the biocontrol potential of the apple tree endophytic microflora.

Summary of the project and main conclusions

It is currently unknown if the latent infection caused by *N. ditissima* consists in an incubation period with no colonisation of plant tissues or represents instead an endophytic phase of the pathogen life-cycle. If demonstrated, the ability of *N. ditissima* to develop in the tree as an endophyte, and to spread within plant tissues at distance from the infection site, would be highly relevant to disease management, currently based on pruning and removal of the infection from the orchard. More importantly, information on the localisation and spread of the fungus within the different plant tissues is required for the development of a sampling and diagnostic procedure, currently unavailable, to detect latent infections.

We tracked the spread of *N. ditissima* in apple trees by performing artificial inoculation of pruning wounds and re-isolation of the pathogen on an artificial growth medium. A high dose of inoculum was applied to fresh pruning cuts performed on trees of four different cultivars (Gala, Discovery, Cox and Golden Delicious). The presence of the pathogen, the distance at which it could be found from the entry wound and the tissues infected (bark or underlying wood) were assessed over time by means of traditional microbiological methods. *N. ditissima* was generally re-isolated, both prior to and after the first canker lesions appeared, from the apparently healthy woody tissue underneath the cambium, at a distance between 10 mm and 40 mm from the pruning wound. It was already known that the fungus can spread longitudinally within the stem of young apple trees, without producing any visible lesion, after the establishment of the first symptoms. Our result additionally showed that, when the entry point is represented by pruning wounds, the pathogen is localised in the internal woody tissues of the branch, at least within the first two months from the onset of infection. These findings represent the first step towards the development of a sampling strategy for the early diagnoses of latent infections, but they need to be complemented by additional information on the spread of the fungus within the plant via different types of wound and during longer periods.

The Enzyme-Linked Immunosorbent Assay (ELISA) technique was successfully used in the past for the detection of *N. ditissima* in symptomatic wooden tissues of apple tree. The ELISA is a quick and straightforward detection test based on the ability of antibodies to detect and bind to a specific target (antigen) in a liquid sample, and on the subsequent visualisation of this interaction by means of an enzymatic reaction which produces a colour change in the

sample. A different, but closely related, technique is that of Lateral Flow Devices (LFD), which are available as diagnostic tools for a number of fungal, bacterial and viral plant diseases. Antibodies are available which can recognise cellular components of *N. ditissima*. We worked on the optimisation of an ELISA assay, which once developed, would provide the cornerstone for the development of a LFD for the European apple canker. Our ELISA was able to detect the pathogen in woody apple tissues (bark and sapwood) even when the microbiological techniques were not successful (**Figure 1**). Results are promising, but further experiments to determine the limit of detection of the technique are necessary to develop a reliable tool.

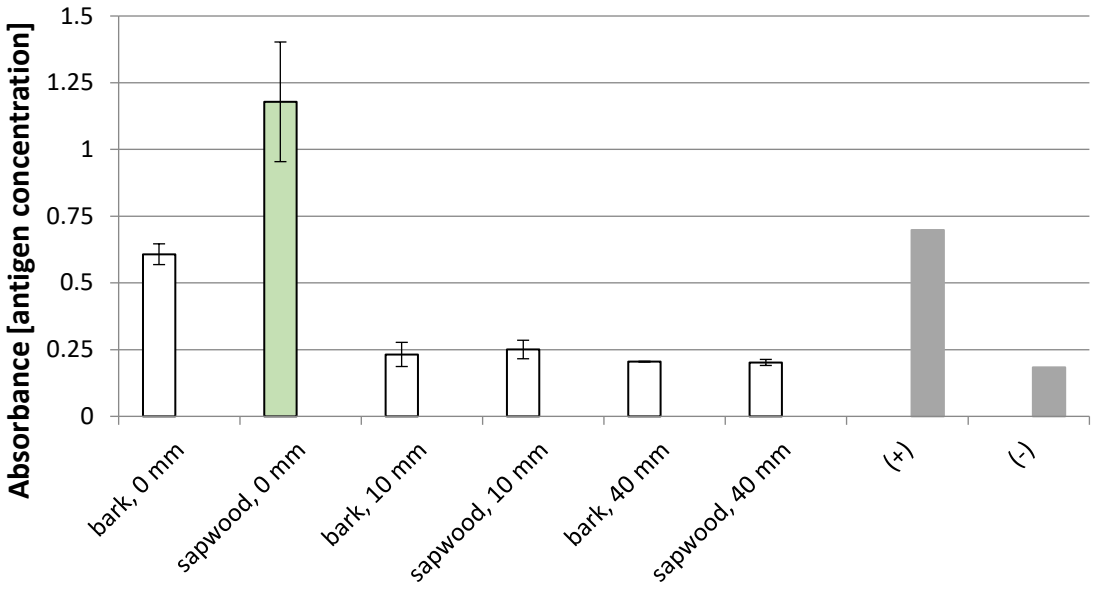

Figure 1 – ELISA on plant material from a natural infected apple branch. The measure of the concentration of *N. ditissima* antigens [absorbance] in bark and sapwood sections at different distances from the canker lesion (0, 10 and 40 mm) is reported. Grey bars correspond to positive (+) and negative (-) antigen. *N. ditissima* could be isolated only from the sapwood at the leading edge of lesion (green bar), but was detected by the ELISA also in the bark of the same branch section.

With the same protocol used to re-isolate *N. ditissima* from inoculated trees, we also collected fungal endophytes from four apple cultivars (Gala, Discovery, Cox and golden Delicious). Endophytes were classified in morphotypes, i.e. in groups based on their morphology on the artificial culture medium. A few of these fungal isolates were identified and their morphotype could be linked to reference species. Among the species identified, there were ubiquitous saprophytes previously found in association with apple leaves (such as *Alternaria* spp., *Epicoccum* spp. and *Cladosporium* spp.). The classification system based on morphotypes showed that the different apple cultivars were characterised by different endophyte profiles.

The next step of this study will involve the analysis of the correlation between the endophyte profiles characterising apple cultivars, showing different field susceptibility to European apple canker, and disease expression.

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 current guidelines published in the Apple Best Practice Guide provide the best advice to achieve effective disease management by means of pruning, chemical control and cultural control. This current project is at an early stage but, based on our preliminary results, some suggestion can be given for the management of infected pruning wounds during the vegetative season:

- As soon as the first canker symptoms appear, branches should be immediately pruned to ensure complete removal of the infection; the minimum recommended distance from the canker lesion at which pruning should be performed is 5 cm
- Paring back of canker lesions should be avoided, and pruning should be performed instead; since the fungus is preferably localised in the sapwood, the removal of the canker lesion in the bark does not ensure removal of the infection.