Grower Summary

CP 161

Understanding endophytes to improve tree health: colonisation of trees by the apple canker fungus and perspectives for biological control

Final report, September 2020

Project title:	Understanding endophytes to improve tree health: colonisation of trees by the apple canker fungus and perspectives for biological control
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The results and conclusions in this report are based on an investigation conducted over a four-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

Headlines

- Internal colonisation of apple trees by the canker fungus, *Neonectria ditissima*, did not occur in the absence of symptoms on the plant, whereas it was observed after canker lesions started to develop.
- Several apple tree-associated microorganisms showed different relative abundance in canker-resistant apple cultivars compared to susceptible cultivars, thus representing interesting candidates for the development of biological control strategies against the pathogen.

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, Kanzi, Jazz, Rubens and Braeburn and the limited availability of registered products for disease control, has resulted in canker becoming more serious in recent years. Moreover, disease management is difficult because after infecting the tree, *N. ditissima* may enter a long latent phase during which no symptoms occur. This represents a serious problem especially in newly established orchards whereby infections which occurred during the propagation phase in the nursery can remain asymptomatic until the young trees are transplanted in the orchard. In these instances, canker lesions typically develop on the main stem or the rootstock, leading to tree grubbing and sometimes causing heavy losses. In some years, over 10% of trees in newly planted orchards can be lost to canker.

The current project set out to address two key areas of research which the industry is interested in:

- developing a diagnostic strategy to test nursery stock material in nursery certification schemes and
- developing alternative control strategies for European apple canker.

Effective diagnosis of a latent infection requires a sensitive and specific detection tool and a targeted sampling strategy. The latter requires an understanding of disease anatomy and of the relationship between the growth of *N. ditissima* in plant tissues and symptom development. These aspects of the pathogen's biology are not fully understood yet. Some observations led to the speculation that the pathogen may extensively spread within the tree during the asymptomatic phase of the infection. Therefore, one of the aims of this present

project was to test the hypothesis that *N. ditissima* can colonise the plant tissues at distance from the site of infection before any visual symptoms can be observed.

Bacterial and fungal biocontrol agents offer ever increasing control options against plant pathogens, which will reduce the reliance on chemical crop protection products. However, until now they have been poorly exploited in relation to European apple canker. Most commercialised biocontrol agents are 'epiphytic', which means they inhabit the surface of bark, leaves and fruits. Their efficacy as pathogen antagonists is strongly dependent on environmental conditions influencing their establishment and survival, such as UV, humidity and temperature. Effective control of European apple canker requires protection of autumnal leaf scars, whose infection can lead to high canker incidence and serious damage to production the following spring. However, environmental conditions at leaf fall are often sub-optimal for the establishment of epiphytic biocontrol agents. Plants also host fungal and bacterial microorganisms associated with their internal tissues, called 'endophytes', which are less affected by environmental factors. It is known that endophytes can interact synergistically or antagonistically with plant pathogens, ultimately affecting disease outcome (incidence and severity). There are differences in the endophyte communities found across different commercial apple cultivars: it is possible that such differences contribute, together with the host genetics, to the different levels of canker resistance observed across cultivars. A second aim of this present project was to compare bacterial and fungal endophytes across canker-resistant and -susceptible cultivars, to identify microorganisms with a biocontrol potential against *N. ditissima*.

Summary of the project and main conclusions

To address the first aim of this project, we studied the ability of *N. ditissima* to grow in the apple tree without producing symptoms. We artificially infected apple trees with a spore suspension of the pathogen via different types of wound (leaf scars and pruning wounds), and then we assessed the distance the pathogen had colonised from the entry point to the healthy-looking part of the branch, both prior to and after canker symptoms had developed.

Firstly, we inoculated shoots on apple trees with a spore suspension of the fungal pathogen via pruning wounds and leaf scars. We used trees of the commercial cultivars Royal Gala and Queen Cox, grown in an orchard at NIAB EMR, East Malling, Kent. Then, we monitored disease incidence over time, and sampled symptomatic and asymptomatic shoots at different time points. Finally, we assessed the localisation of the pathogen at the entry site, i.e. the asymptomatic or cankered infected wound on asymptomatic or symptomatic shoots, respectively, as well as at several distances along the shoot. In the case of leaf scar inoculations, samples were taken from the infection point and up to a distance of 10-15 mm,

whilst in the pruning wound inoculations samples were collected from the inoculation point and up to a distance of 40 mm. We used a highly specific DNA-based method to detect *N. ditissima*-specific DNA in plant samples. Our findings suggested that:

1. *N. ditissima* can remain latent for several months in the infected wounds before cankers are formed; notably, we found latent infections up to four and seven months after the inoculation in pruning wounds and leaf scars, respectively.
2. During such latent period, the pathogen does not spread in the tree, but it remains confined at the infected wound.
3. However, once visual symptoms occur, *N. ditissima* can grow beyond the limits of the canker lesion into the adjacent, apparently healthy portions of stem and branches; in this study, the pathogen was detected up to 15 mm from the edge of the symptomatic tissue.

To address the second aim of this project, we studied the bacterial and fungal endophytes associated with the apple tree. Notably, we assessed how the scion cultivar, the rootstock cultivar and the site of planting determine the diversity of endophytes, and we compared endophytes associated with different scion cultivars differing in their respective canker-resistance. We focussed on the endophytes which are found at the leaf scar, as this the main natural infection route for the pathogen.

Eight scion cultivars were grafted onto two different rootstocks at a common nursery (Frank P Matthews), then planted in two orchards in Kent. Samples were collected from leaf scars of young shoots in autumn 2018. A DNA-based method (Next Generation Sequencing, NGS) was used to determine fungal and bacterial endophytes and their relative abundance in different samples. Our findings suggest that:

1. Overall, apple tree endophytes were mainly influenced by the site of planting and by the scion cultivar.
2. Some endophytes displayed higher or lower abundance across cultivars differing in canker-resistance.

The different relative abundance of some endophytes indicates that they may act as antagonists or facilitators of the pathogen and thus reduce or aggravate, respectively, disease incidence and severity. Based on these findings, further studies are being carried out (BBSRC project BB/P007899/1) to investigate their potential and how this can be exploited against European apple canker. Moreover, our results suggest that orchard management practices and cultivation history may influence the diversity of endophyte associated with apple. In fact, different plant species may host different endophyte populations, which contribute to build-up

of environmental inoculum that can in turn colonise apple trees. Similarly, it is known that specific plant hosts can act as *N. ditissima* reservoir, thus acting as pathogen inoculum source. Therefore, practices such as weed control and hedgerow management may help to achieve successful manipulation of endophytes, as well as effective disease control, by shaping the local microbial inoculum. Our results pave the way for the development of alternative strategies for European apple canker control.

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 greatly reduce the additional expense required to replace diseased trees.

Action points for growers

Successful European apple canker management needs to combine different approaches including systematic pruning of cankers, removal of heavily infected trees and timely fungicide applications. Removal of canker lesions is essential to achieve efficient disease control, as it reduces the source of infection in the orchard.

- Pruning should be performed as soon as cankers become visible on the plant. In fact, once symptoms appear the pathogen might spread internally in shoots and branches and reach healthy parts of the tree.
- To account for the possibility of internal spread of the pathogen, pruning cuts to remove canker lesions should be performed at a minimum distance of 10 cm from the canker edge.
- Paring off cankered bark on main stem or scaffold branches is not recommended, as the pathogen may survive in the underlying wood and produce a new canker lesion; pruning should be performed instead.