Project title:	The role of endophytes in affecting European canker development on apple
Project number:	TF 226
Project leader:	Professor Xiangming Xu NIAB EMR
Report:	Annual, October 2018
Previous report:	N/A
Key staff:	Dr Matevz Papp-Rupar (NIAB EMR) Dr Louisa Robinson-Boyer (NIAB EMR) Dr Lucas Shuttleworth (NIAB EMR) Dr Tom Passey (NIAB EMR)
Location of project:	NIAB EMR, East Malling, and several farms in Kent
Industry Representative:	Peter Checkley
Date project commenced:	1 October 2017
Date project completed (or expected completion date):	30 September 2021

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

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.

Xiangming Xu

Head of Pest & Pathogen Ecology Department

NIAB EMR

Signature  Date: October 2018

Report authorised by:

Xiangming Xu

Head of Pest & Pathogen Ecology Department

NIAB EMR

Signature  Date: October 2018

Contents

GROWER SUMMARY

Headline

- Studies have been initiated to determine the role of microbial endophytes in the control of *Neonectria ditissima* (the cause of apple canker).

Background and expected deliverables

European canker, caused by *Neonectria ditissima*, has become the most damaging disease of apple in recent years across all major apple growing regions worldwide. Modern cultivars lack effective resistance to this pathogen and in Europe most efficacious methods of chemical control are no longer available. Cultivars differ in their susceptibility but there is no absolute resistance. Previous work, conducted at NIAB EMR, has demonstrated that asymptomatic infection in nursery trees is a significant source of the disease in production orchards. The most economically important damage occurs when the nursery-borne latent infection becomes active and develops into canker on the main trunk during orchard establishment (within three years of planting) – leading to tree death. Ample empirical evidence suggests that stresses following planting can promote symptom expression of those nursery-borne latent infections.

An endophyte is a microbe that lives within a plant for at least part of its life cycle without causing apparent disease. Endophytes have been found in all species of plants studied to date although the endophyte/plant relationships are not well understood. Certain microbial endophytes can help plants to tolerate biotic stress, such as attacks by plant pathogens and herbivory, or abiotic stresses, including salt, drought or heat stresses. It has been shown in numerous host species that recruitment of specific microbes into the rhizosphere is partially under host genetic control and there is increasing evidence that host genetics influence the microbes occupying the endophytic niche. Endophyte composition can also be influenced by pathogen presence and crop management practices. Current research focuses on how we could exploit endophytes to produce crops that grow faster and are more resistant and hardier than crops lacking endophytes.

Recently, we have obtained preliminary data showing a link between antagonist fungal endophytes and cultivar tolerance to this pathogen. One fungal endophyte group, identified as belonging to the genus *Epicoccum* (most likely as *E. purpurascens*, previously known as *E. nigrum*), is much more abundant in two canker-tolerant cultivars than in two canker susceptible cultivars. *Epicoccum purpurascens* is a known antagonist against *Monilinia laxa* (causing stone fruit brown rot) and is being commercially exploited for control of brown rot on stone fruit. It is natural, therefore, to speculate whether the abundance of *E. purpurascens* is related to tolerance to canker development and, if so, whether we could exploit *E. purpurascens* for canker management.

In this LINK project, we aim to build on the preliminary data to investigate whether cultivar differences in tolerance to *N. ditissima* are associated with specific endophytes and, if so, identify the organism(s) and conduct further *in vitro* and *in vivo* biocontrol assays to assess specific endophytes against *N. ditissima*. As well as the direct effect against the canker pathogen we shall study whether these specific endophytes could reduce canker development via inducing host defence systems against the pathogen. To improve breeding for canker resistance, we shall determine to what extent the recruitment of specific endophytes is genetically controlled by hosts by mapping QTLs (quantitative trait loci) and to determine the

extent of overlaps of these QTLs with those mapped for canker resistance. Then we will conduct experiments to assess (1) to what extent recruitment of endophytes is influenced by soil characteristics and host genotypes, and (2) whether canker symptom expression is related to planting times and the abundance of specific endophytes across a number of orchards. Finally, to assist in canker management, we will investigate the extent to which endophyte profiles of a specific apple genotype can be influenced by management practices (irrigation and soil amendment).

Summary of the project and main conclusions in Year 1

We have successfully initiated all experimental studies on time.

(1) We established two orchards to study whether specific endophytes are associated with canker resistance/susceptibility. Eight cultivars of differing canker susceptibility were grafted to two rootstocks (one susceptible and the other resistant to canker) and the trees were planted in the two orchards in spring 2018. Endophytes at the leaf scar position will be determined in 2018/2019.

(2) We have conducted an *in vitro* test that confirmed that one specific *E. purpurascens* strain (isolated from an apple tree in the UK) can significantly reduce colony expansion of the canker pathogen. We isolated over 47 endophytes from apple trees and will select a number of isolates to further test their effects against the canker pathogen.

(3) We have initiated studies to investigate whether we could externally apply *E. purpurascens* to increase its concentration *in planta* and to assess whether this will affect canker development. M9 rootstock plants in stoolbeds were first inoculated with *E. purpurascens* and a few months later with the canker pathogen at leaf fall. These plants will be monitored for canker development as well as for abundance of the applied endophyte.

(4) We have set up an experiment studying whether soil amendment with AMF and/or PGPR under two irrigation regimes (well-watered and deficit irrigation) can affect plant endophytes composition, tree health and canker development. Samples were collected and are being assessed for various traits.

(5) Nearly 2,000 trees are being raised at the FP Matthews nursery for two field experiments. One set of trees will be planted in the 2018/19 winter at one site to study whether application of an endophyte could induce plant defence responses and lead to reduced canker development. Trees were inoculated with *E. purpurascens* in September 2018. The second set of trees will be planted in the late autumn 2018 and early spring 2019 at three sites to study whether soil physical and biological properties, cultivars, and planting dates affect endophyte populations and canker expression. All trees will be inoculated with the canker pathogen in early November 2018.

Financial benefits

- The project is in its infancy and it is too early to quantify financial benefits for growers.

Action points for growers

- At this stage of the project, there are no action points to recommend to growers.

SCIENCE SECTION

Background

European apple canker, caused by *Neonectria ditissima*, is a destructive disease of apple trees and current methods of control, based on protective fungicides, are only partially effective, non-sustainable, and to date reliant on copper-based fungicides which are no longer permitted. This pathogen has a complex lifecycle with all year-round potential of producing ascospores and conidia, which infect wounds (e.g. leaf scars and pruning cuts). The pathogen also infects fruit, leading to losses in store as a post-harvest rot. The most damaging phase of the disease is the canker on the main trunk of a young tree in newly established orchards. Most of these cankers result from infection in nurseries but remain latent until post-planting in orchards. Modern nurseries are high input operations with fungicide, nutrients and water added to encourage vigorous growth in the first two years. A nursery tree is made up of two components; a rootstock, harvested from a stool bed, and a scion, harvested from a 'mother tree', both sources can harbour latent infection which is masked by the high inputs through the nursery phase but then expressed during the establishment stage in the orchard where the tree experiences abiotic and biotic stress. This is exacerbated in modern intensive fruit wall orchard systems (c. 3000 trees/ha) where the trees are much smaller than in traditional orchards coupled with the varieties (e.g. Gala, Rubens, Jazz and Kanzi) being much more susceptible, resulting in a high incidence of tree death from trunk cankers during orchard establishment. Tree death due to canker of over 10% is common during orchard establishment for susceptible varieties (Saville, unpublished). Past experience has shown that canker symptom expression in newly established orchards is related to particular sites.

Neonectria ditissima is a wound pathogen and accordingly absolute host resistance has not been observed. However, quantitative differences have been determined between genotypes in their response to this pathogen. There is currently a concerted effort in Europe (UK/NL/SWE) and New Zealand to determine the underlying mechanisms of this resistance/susceptibility in order to breed for increased tolerance/resistance to the pathogen. Breeding apple cultivars requires a minimum of 15-20 years. Yet there is an urgent need to understand the biology of this disease to develop better management strategies in the medium term.

Endophytes associated with specific apple genotypes may be an important component affecting latent canker development, thereby contributing to field resistance. Recent evidence suggests that endophytes may induce plant defence responses, produce secondary metabolites that inhibit pathogens, directly compete with invading pathogens or a combination thereof. Resistance to Dutch elm disease (*Ophiostoma novo-ulmi*) is associated with reduced diversity in fungal endophytes in the host. Endophytes of woody angiosperms were shown to play an important role in host defence. The endophytic fungus *Muscodor albus*, originally isolated from *Cinnamomum zeylanicum*, produces a mixture of volatile organic compounds in culture which have a wide spectrum of antimicrobial activity. Endophytes can also help plants tolerate abiotic stresses, e.g. salt and heat tolerance. Recently, it has been demonstrated that a fungal endophyte (*Piriformspora indica*) enhanced its host plant's (rice) tolerance to root herbivory through changes in gibberellin and jasmonate signalling. It has been shown in numerous host species that recruitment of specific microbes into the rhizosphere is partially under host genetic control and there is increasing evidence that host genetics influence the microbes occupying the endophytic niche. Endophyte composition can also be influenced by pathogen presence, production system, and AMF colonisation.

Recently we obtained preliminary data (see below) in an unfunded project, which suggests that specific endophytes may be associated with cultivar differences in their susceptibility to *N. ditissima*. Orchard-specific factors can greatly influence relative abundance of endophytes, which in turn, may affect canker symptom expression. We hypothesise that endophytes play a role in affecting canker expression of latent infections.

Orchard-specific factors (abiotic – soil type, soil water deficit, nutrient supply; and biotic – soil microbial population, including AMF and PGPR) may indirectly influence canker symptom expression via their effects on the endophytic profile (identity or abundance) or via induction of host defence responses. Plants respond to multiple stresses differently from how they do to individual abiotic and biotic stresses, activating a specific programme of gene expression relating to the exact environmental conditions encountered. AMF and PGPR can induce specific plant defence responses. Plant hormones are major components of those pathways and regulate differential defence responses to specific types of attackers. Broadly, jasmonic acid (JA) and ethylene (ET) are responsible for elicitation of defences against necrotrophic pathogens, whereas salicylic acid (SA) is predominantly involved in defence against biotrophic pathogens. The SA- and JA-pathways can exhibit negative crosstalk - *N. ditissima* is classified as a necrotrophic pathogen; hence increased defence signalling (SA) against biotrophic pathogens (induced by external factors) may be at the expense of reduced defence against colonisation by latent infections of *N. ditissima*. Simultaneous occurrence of biotic and abiotic stresses can cause either a positive or negative plant defence response to a would-be-pathogen. This interaction between biotic and abiotic stresses is orchestrated by hormone signalling pathways, in particular abscisic acid (ABA). We hypothesise that the negative crosstalk in plant hormone signalling in response to external factors (e.g. soil water deficit, AMF, PGPR) leads to accelerated development of *N. ditissima* latent infection.

Overall objectives

The overall objective is to assess the role of endophytes in conferring resistance to *N. ditissima*, and to assess how the abundance of the specific endophytes is influenced by other biotic/abiotic factors in relation to plant defence responses and canker development. Project outcomes will underpin the development of practical measures to reduce canker development, particularly in the early stage of orchard establishment. This will not only reduce tree death in the early establishment phase but also result in reduced secondary infection of braches and fruit due to a reduction in inoculum.

Specific objectives include: (1) confirming the association of specific endophytes with cultivar tolerance to *N. ditissima*; (2) quantifying biocontrol potential of specific endophytes that showed differential abundance between susceptible and resistant cultivars; (3) investigating whether specific endophytes induce host defence responses that may contribute to reduced canker development, (4) mapping QTLs that control recruitment of specific endophytes; (5) determining the extent to which the abundance of specific endophytes can be influenced by single or combined biotic/abiotic factors, (6) conducting a 'common garden experiment' to determine the extent of association of canker symptom development with endophytes and other abiotic/biotic factors.

In addition to funding from BBSRC and AHDB, the following industry partners also provide in-kind support: **Adrian Scripps Limited**, **Avalon Produce Limited, ENZA** (T&G global subsidiary), **Frank P Matthews Limited,** and **Worldwide Fruit Limited.**

The entire project is divided into six work packages, each dealing with specific topics. In this report, in order to have a better flow of information, we report work package by work package.

WP1: Endophyte profiling

In a pilot study (Saville and Xu, 2016, unpublished data) showed that the endophyte profile, as determined by Illumina next generation sequencing, is statistically ($P<0.05$) distinct in canker-susceptible and resistant cultivars (Figure 1).

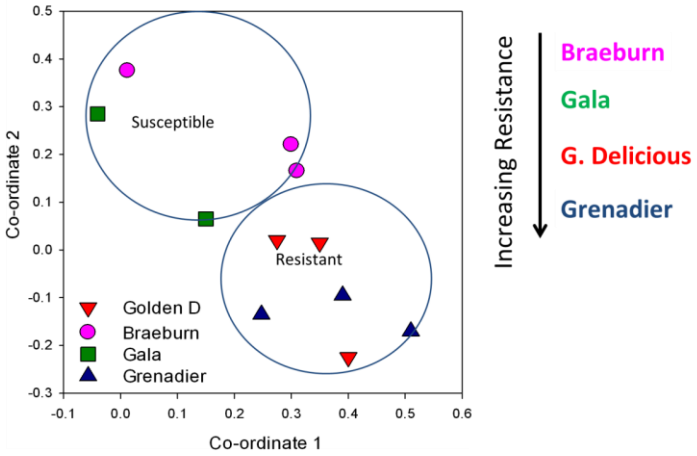

Figure 1. The endophyte profile, as determined by Illumina next generation sequencing, is distinct in two canker-susceptible and two resistant cultivars

Objectives

1. To determine whether there are differences between endophyte populations in leaf scars on 1-year-old shoots among a number of varieties and whether such differences are associated with the cultivar susceptibility to European apple canker
2. To determine whether rootstocks and environmental conditions can affect endophyte populations

We chose to profile endophytes in the leaf scars because leaf scars are one of main natural entry points for canker infection under field conditions.

Material and methods

Eight scion cultivars were grafted onto two rootstocks, and the trees were planted in two sites in the spring 2018 (Table 1, Figures 2-3). Of the eight cultivars, three were resistant and the others susceptible to canker. M116 rootstock is believed to show resistance against canker whereas M9 is susceptible. For each scion/rootstock combination, there were 15 trees at each site; trees were planted in a randomised block design of eight blocks: 7 blocks of pairs, 1 block of single.

We sampled 1-year-old shoots just before leaf fall on 10th and 12th of October 2018 at the Perry Street and Friday Street Farm, respectively. There were five biological replicates for each scion/rootstock combination, no more than one from each block at each site, giving 80 samples per site. One year old wood with leaves still attached were collected from the leader and up to 4 feathers from each tree and taken to the lab.

In the lab, leaves were removed in a laminar flow hood to expose the leaf scar tissue. We did not remove epiphytic cells on the bark surface because:

1. Once the leaf has been removed, surface sterilisation also affects internal tissues of the leaf scar and change endophytes
2. Epiphytic microorganisms at the leaf scar can also affect infection and disease expression.

Instead, leaf scar tissue with minimal amount of bark was carefully dissected with a sterile scalpel. A total of 12 leaf scars (ca. 0.3 g) per tree were dissected from 3 – 5 shoot sections, pooled and stored at -80 °C until DNA extraction, constituting one biological sample.

These samples will be extracted for DNA and subjected to next generation sequencing (NGS) technology (DNA meta-barcoding) to profile both bacterial and fungal populations for each sample.

Table 1. Summary of WP1 experimental planting design. Cultivars that are believed to be resistant against canker are shown in green, and susceptible in red.

Scions	Rootstocks	Planting sites	Total trees
1. Robusta 5 2. Golden Delicious 3. Grenadier 4. Gala 5. Braeburn 6. Jazz 7. Kanzi 8. Rubens	1) M116 2) M9 337	1) Friday St Farm: Friday St, Maidstone, ME17 3DD 2) Perry Farm: Perry Lane, Wingham, CT3 1EQ	15 trees per scion/rootstock combination (240) planted in two sites (total = 480)

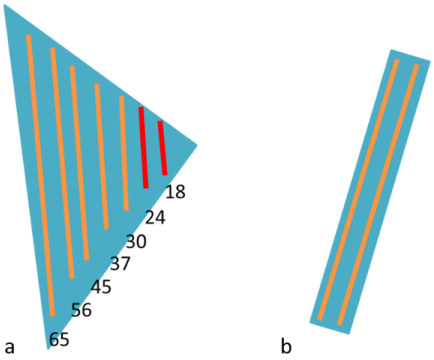

Figure 2. Planting sites layout: (a) Friday St Farm, 275 tree stations; (b) Perry Farm, 290 tree stations. Trees in the two short rows (red) were extra trees and may not be required for sampling.

Figure 3. Planting WP1 trees at Perry Lane, Wingham (12/08/2018)

Results

DNA extraction and meta-barcoding are planned for winter 2018/19 and the first results should be available by June 2019.

WP2: Endophyte biocontrol efficacy

In a preliminary meta-barcoding study, we identified several fungal endophytes that are significantly more abundant in canker resistant apple cultivars than in susceptible cultivars. One of them, *Epicoccum purpurascens* (previously known as *Epicoccum nigrum*), has been previously shown to have biocontrol properties against several *Fusarium* spp. (Ogórek and Plaskowska, 2011), *Pythium* damping-off in cotton (Hashem and Ali, 2004) and *Monilinia* spp. brown rot in peaches (De Cal *et al*., 2009; Larena *et al*., 2004).

Objectives

1. To assess whether *Epicoccum purpurascens* could control *Neonectria ditissima in vitro* and *in vivo*
2. To determine whether there are other apples endophytes with biocontrol potential present in local apple trees

Material and methods

Identification and isolation of endophytes: Sections of 1-year- old wood from cultivars Royal Gala and Cox (both susceptible to cankers) were collected from the Wisemans orchard at NIAB EMR in the autumn 2017. Wood sections were surface sterilised and plated on PDA plates, containing antibiotic rifampicin and one fungicide to prevent the growth of bacterial and fungal species belonging to genus *Botrytis*, *Alternaria* and *Penicillium*. In total, we obtained 47 pure fungal cultures through a series of sub-culturing. These pure cultures were then characterised for colony, hyphae and spore morphology and sequenced for the ITS2 region for identification. Based on the literature, we selected a second fungal endophyte, *Gliocladium roseum*, for *in vitro* assay against *N. ditissima*.

In vitro challenge assay: We conducted an *in vitro* challenge assay to assess biocontrol potential of a UK isolate of *E. purpurascens* B14-1 against a UK *N. ditissima* isolate R28/15. Ten PDA plates were used for the challenge assay and another four plates for controls (using *N. ditissima* only). On the bottom of all plates, a 6 cm line was drawn across the middle and labelled with E (for *Epicoccum*) at one end and N (for *Neonectria*) at the other. This line was used to measure the growth of each fungal colony across the plate.

6 mm agar plugs of *N. ditissima* and *E. purpurascens* were placed at opposite ends of the 6 cm line on the plate with mycelium side down. Plates were then incubated the right way up in the dark at 20°C. Once fungal growth started, the plates were turned upside down to reduce the risk of condensation causing contamination. *Neonectria ditissima* colony size across the line on the plate was recorded twice a week for several weeks.

The same experimental setup was used for the *in vitro* assay of *G. roseum*, another fungal endophyte isolated at NIAB EMR.

Assessing biocontrol efficacy of *E. purpurascens in planta*: A large amount of *E. purpurascens* B14-1 conidia were produced in solid state fermentation in sterile vermiculite/lentil meal media in solid bioreactor bags (Larena *et al*. 2004; **Error! Reference source not found.**4). After 3-4 weeks of incubation, we harvested conidia by shaking media with water. Conidial suspension was filtered through several layers of muslin and concentrated by centrifugation. In mid-July 2018, stool beds of M9 rootstocks were inoculated with *E. purpurascens* conidia. Soil around the plants was pre-wetted with a watering can. Three different conidial application methods were tested; spray, root drench and spray + drench. The spray was applied by large hand-held sprayers to run-off. Spore concentrations used:

1. Spray = ca. 238500 conidia/ml, 10 ml/plant = ca. 2385000 conidia/plant
2. Drench = ca. 23850 conidia /ml, 100 ml/plant = ca. 2385000 conidia/plant

Four 5 m long stoolbed rows were used as 4 experimental blocks (Figure 5).

In late-October 2018 (leaf fall), leaves of all shoots were all removed by hand in all the plots and exposed leaf scars were inoculated with *N. ditissima* conidia at 10^4 conidia per ml. The conidial suspension was sprayed with a knapsack sprayer at the rate of 1 L per 5m block.

In spring 2019, canker symptoms will be scored in all blocks to assess *in-planta* biocontrol efficacy of *E. purpurascens*.

Figure 4. Solid state fermenter bags with vermiculite/lentil meal media for *E. purpurascens* conidia production

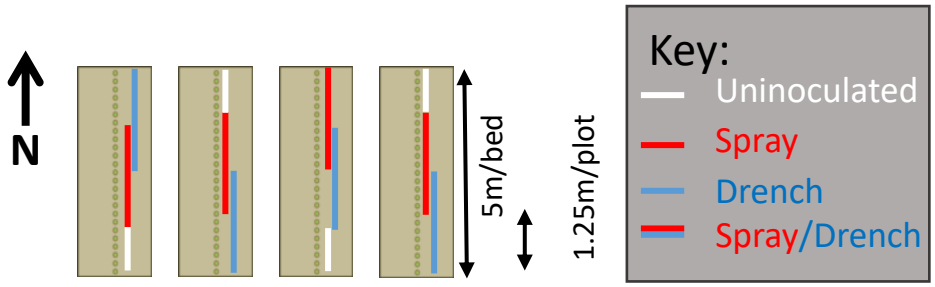

Figure 5. Epicoccum inoculation of M9 rootstock stool beds - experiment set-up

Results

Epicoccum purpurascens B14-1 has significantly reduced the growth of *N. ditissima* in the *in vitro* challenge assay from day 13 onwards (Figure 6). The observation of clear zone between *N. ditissima* and *E. purpurascens* on the plates (Figure 7) indicated that antibiotic compounds could be secreted in the media preventing the growth of *N. ditissima*. Reduction in the growth of *N. ditissima* in the present experimental conditions could be in part due to the faster growth of *E. purpurascens* as well. *In vitro* results prompted us to move forward and test if *E. purpurascens* can reduce *N. ditissima* symptom development *in planta*.

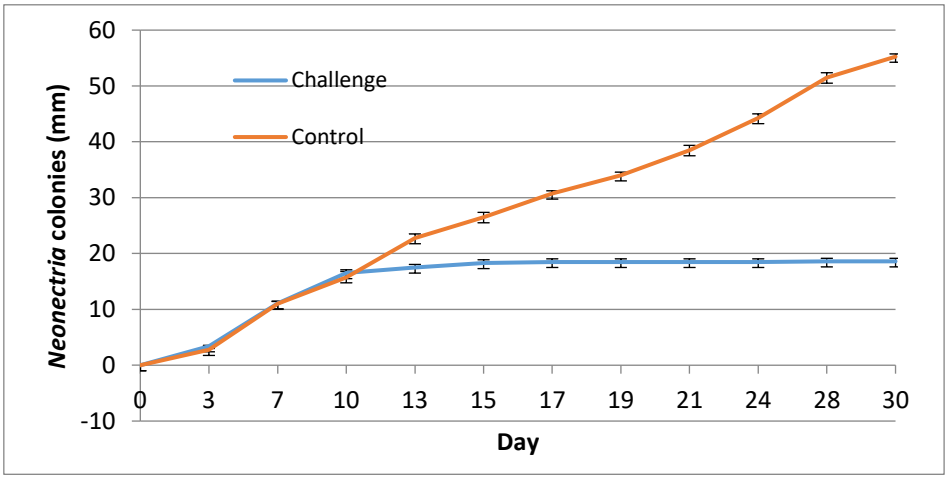

Figure 6. Growth of Neonectria ditissima Hg199 colonies in a challenge assay against *Epicoccum purpurascens* B14-1 grown on PDA plates.

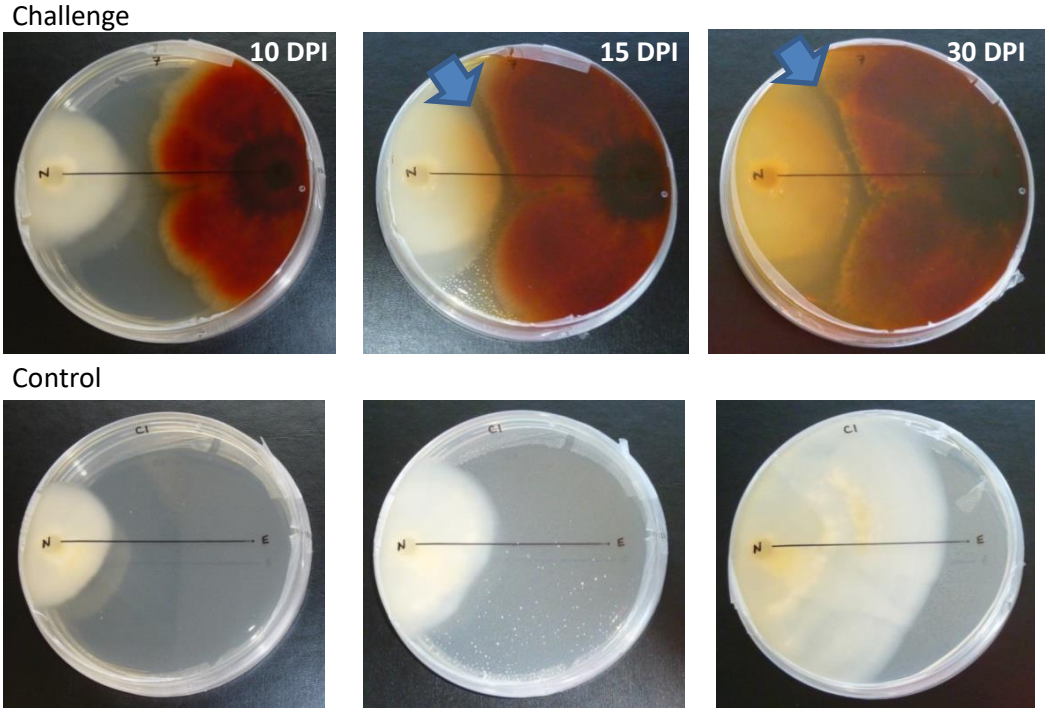

Figure 7. Example of *N. ditissim*a growth on PDA plates challenged with *E. purpurascens* (top) and control plates (bottom) at 10, 15 and 30 day post inoculation (DPI). Clear zone indicating possible secretion of antibiotic compounds by *E. purpurascens* is indicated with blue arrows

Gliocladium roseum, which has been shown to have biocontrol properties against *Botrytis cinerea* in strawberries (Cota *et al*., 2008), did not show direct biocontrol effect against the *in vitro* growth of *N. ditissima* (Figure 8). The *N. ditissima* colonies grew at the same rate regardless whether they were grown alone or together with *G. roseum* (data not shown). *Gliocladium roseum* grew over *N. ditissima* without any observable interaction (Figure 8, bottom panel).

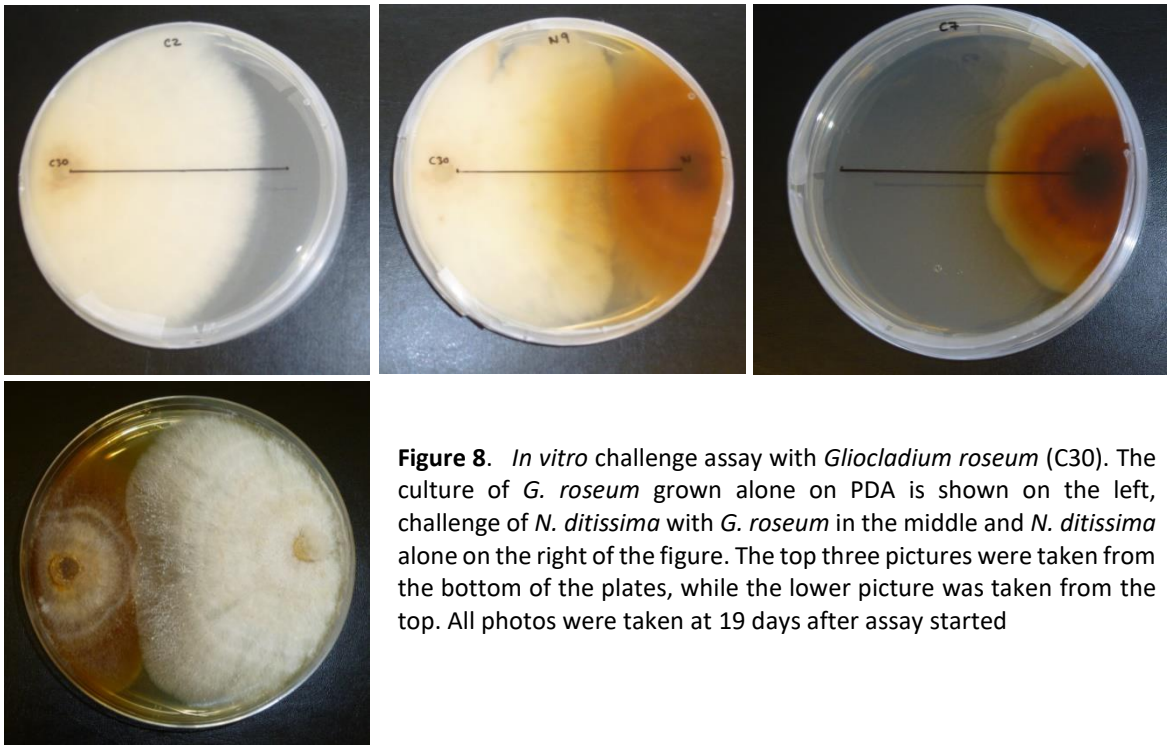

Figure 8. *In vitro* challenge assay with *Gliocladium roseum* (C30). The culture of *G. roseum* grown alone on PDA is shown on the left, challenge of *N. ditissima* with *G. roseum* in the middle and *N. ditissima* alone on the right of the figure. The top three pictures were taken from the bottom of the plates, while the lower picture was taken from the top. All photos were taken at 19 days after assay started

The endophyte collection (Figure 9) contains several isolates of *Aureobasidium* spp.; *A. pullulans* can control *B. cinerea*, *Colletotrichum acutatum* (bitter rot) or *P. expansum* (blue mould) in apples postharvest. Further assay will be conducted in the winter 2018/19.

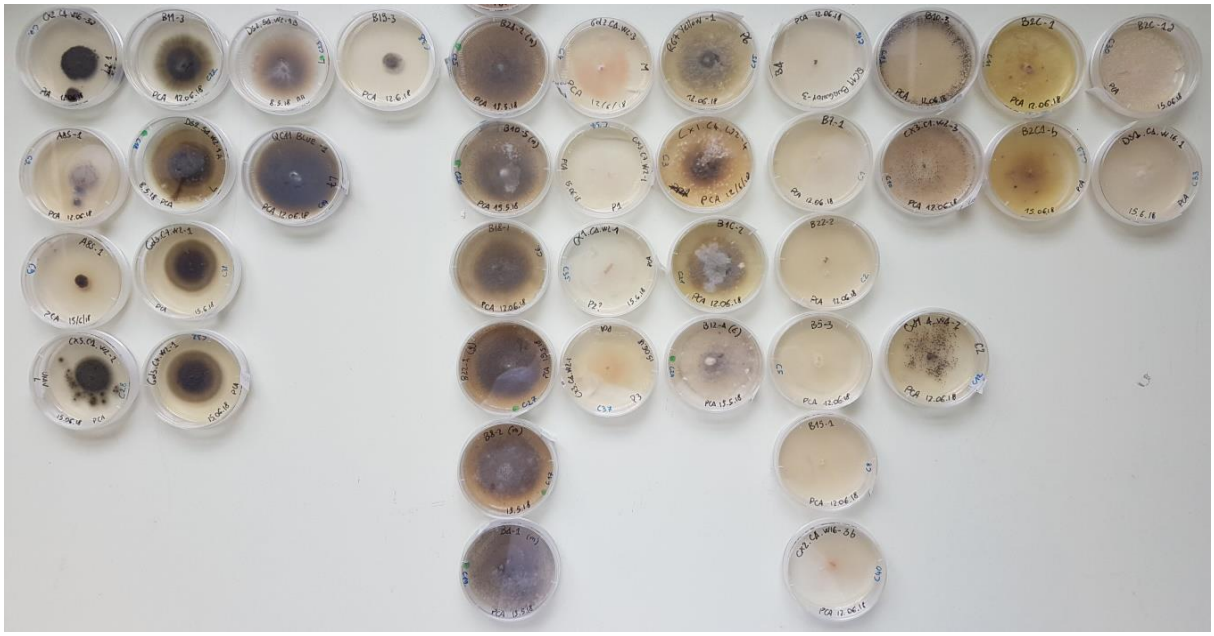

Figure 9. The different morpho-types present in the apple fungal endophyte collection

WP3: Induction of plant defence response by endophytes

Objectives

1. To determine how apple trees respond to endophytes, specifically whether endophytes induce plant defence responses

Material and methods

The following general experimental approach has been used. Candidate endophytes are to be inoculated onto two cultivars Kanzi (susceptible) and Golden Delicious (resistant) by foliar spray. There will be four treatments: inoculation with *N. ditissima* (yes, no) x inoculation with *E. purpurascens* (yes, no). Currently, 120 Kanzi and 120 Golden Delicious trees are grown at FP Matthew's nursery; 30 replicate trees per treatment. Expression of specific defence genes in SA (ICS119) and JA (JA-Ile synthase OsJAR17) pathways will be quantified using qRT-PCR methods based on published protocols. SA and JA concentrations will also be measured using vacuum extraction of xylem sap.

Production of *E. purpurascens* B14-1 conidia was as described previously (WP2) in 2 L solid fermenters on sterile vermiculite/lentil meal mix. Trees were inoculated at the FP Matthew's nursery site on 7 September 2018 after 5 pm (Figure 10) to ensure high humidity after inoculation. Soil around of the base of the trees was pre-wetted by watering. Each tree received: (1) root drench of $1.2x10^6$ conidia (200 ml conidial suspension) and (2) foliar spray of $3.6x10^6$ conidia (ca. 120 ml conidial suspension).

These trees will be inoculated with *N. ditissima* in the early November 2018, before the trees are lifted and stored. Foliar spray at 10^4 conidia will be used.

The trees will be lifted early December 2018 and planted at a site provided by Scripps Ltd in the winter 2018/19. Trees will be sampled for qPCR analysis of those specific genes and hormone analysis one, three and five months after canker inoculation; five trees per cultivar will be sampled on each date. Canker symptoms will be assessed on the remaining 15 trees per treatment for at least 12 months after *N. ditissima* inoculation.

Figure 4. Inoculation of *E. purpurascens* at FP Matthews' nursery in September 2018. Trees were marked with orange tags, drenched and sprayed with *E. purpurascens* conidial suspension

WP4: Mapping QTLs responsible for recruiting endophytes

Objectives

1. To map QTLs responsible for recruiting specific endophytes that have biocontrol potential against nectria canker
2. To assess the overlap of these QTLs with those mapped for canker resistance in the same mapping progeny

Results

This WP will start in the autumn 2019.

WP5: Effects of specific factors on endophytes

Endophytes associated with specific apple genotypes may be an important component affecting latent canker development, thereby contributing to field resistance. Recent evidence suggests that endophytes may induce plant defence responses, produce secondary metabolites that inhibit pathogens, directly compete with invading pathogens or a combination thereof (Arnold *et al*., 2003). Endophytes can also help plants tolerate abiotic stresses, e.g. salt and heat tolerance (Rodriguez *et al*., 2008). Endophyte composition can also be influenced by pathogen presence, production system (Agler *et al*., 2016), and AMF colonisation (Liu *et al*., 2011 and Wearn *et al*., 2012)

Objectives

1. To evaluate biological soil amendments [arbuscular mycorrhiza (AMF) and plant growth promoting rhizobacteria (PGPR)] for their effects on tree health and canker expression
2. To investigate the effects of deficit irrigation and AMF/PGPR on endophyte profiles and canker expression.

Material and methods

Gala trees on M9 feathered maidens (code R02S04) were potted into 10 L pots with Halstone Top soil (Travis Perkins); top soil was used as it contains natural-soil-occurring microbes. Pots were placed on saucers to prevent cross contamination of AMF/PGPR and for easy assessment of whether excessive irrigation was applied. Trees were grown in a polytunnel at NIAB EMR.

At planting, appropriate soil amendment (one of the four amendment treatments:

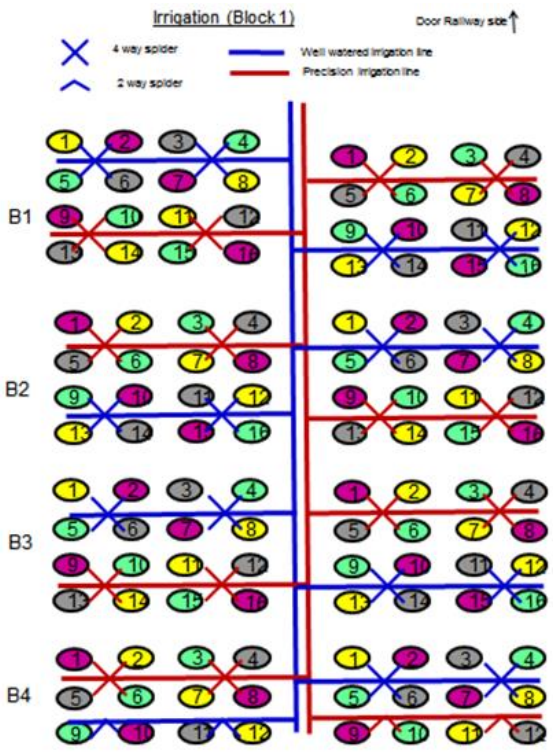

Figure 51. Randomised block design for trees grown with four soil amendment treatments and two water regimes

AMF, PGPR, PGPR+AMF and Control) was applied. Trees were then grown for six weeks prior to the irrigation treatment being applied; this was necessary to have uniform tree establishment. Two water regimes, well-watered (WW) and 65% RDI (regulated deficit irrigation), were applied. A total of 120 trees were arranged in a randomised block design (Figure 11). There are 30 replicate trees for each of the four amendment treatments - 15 for each irrigation regime.

To ensure the WW trees were watered to the capacity, tree water use was estimated weekly:

1. Two trees from each of the four treatments subjected to the WW treatment were chosen throughout the polytunnel and weighed on two consecutive days.
2. Tree and pot weights were recorded directly after an irrigation event on day 1, and the dripper removed from the pot.
3. Exactly 24 hours later, the tree was re-weighed and water loss calculated to calculate water usage over a 24 hour period.

The estimated water use was then used to schedule irrigation the following week.

We regularly measured plant response to RDI, stomatal conductance, and photosynthesis using a stomatal conductance meter (Figure 12). In addition, stomatal conductance and photosynthesis were measured at six time points throughout the day on three occasions. Soil matrix potential was also measured using a soil moisture probe weekly.

Figure 6. Experiment set up in the poly tunnel (left) to study plant responses to AMF and PGPR amendments, and measuring stomatal conductance (right)

All treatments were sampled on three time points: 1, 3 and 5 months after the irrigation treatment began. At each time point, eight trees from each treatment – irrigation/amendment (one from each block) were destructively harvested. Leaf samples were collected for ABA, JA and SA hormone analysis. Roots will be analysed for endophytes using fungal ITS and bacterial 18S primers using the NGS technology.

Results

Trees were established in 2018 and samples were collected from the first two sampling points. The final sampling point will be collected in December 2018. Samples are stored for leaf hormone analysis, and root and wood samples have been collected for endophyte analysis. Roots were collected and stained for the detection of AMF in inoculated trees. Levels of AMF

were high across all treatments (Figure 13), indicating the top soil has a high level of resident AMF inoculum.

Stomatal conductance and photosynthesis were recorded throughout the growing season. The results were highly variable across treatment conditions (Figure 14); the nature of these variable data is most likely to result from the exceptional hot weather encountered during the summer of 2018.

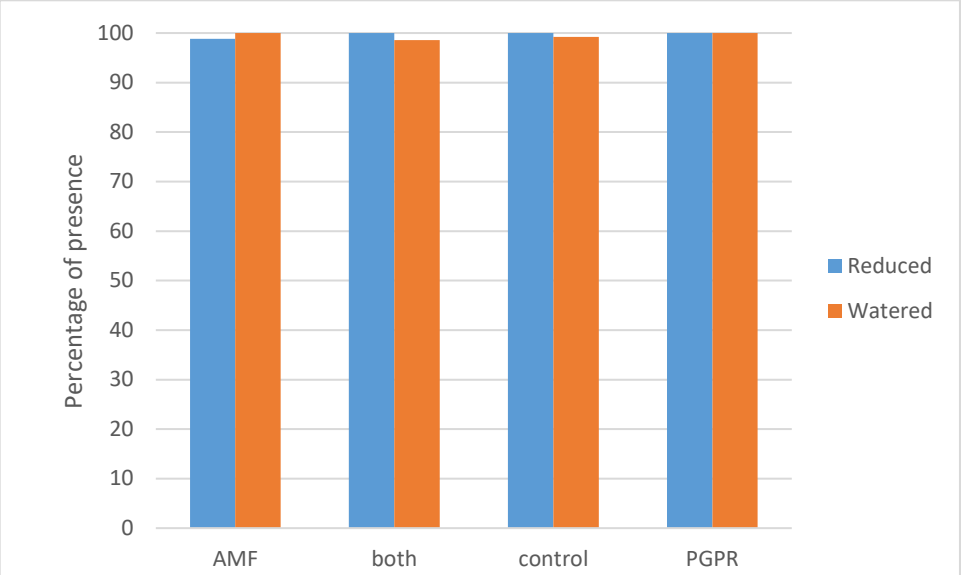

Figure 7. Percentage of apple root fragments with AMF

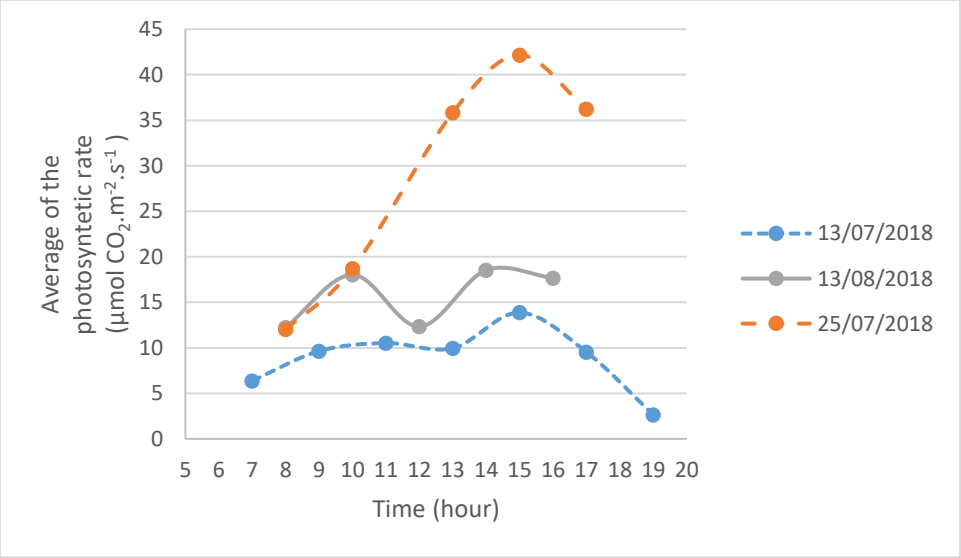

Figure 8. Examples of average photosynthesis rates during daytimes

WP6: Common garden experiment

Although there is ample evidence suggesting that there are specific sites which are particularly prone to canker expression, to date no research has been conducted to identify which specific factor(s) that could be responsible for promoting canker symptom development. Most importantly, empirical evidence suggests that lengthening storage time of trees between lifting and planting would worsen canker development in orchards. We propose to conduct a 'common garden experiment' to obtain a large dataset for establishing statistical association

between canker expression, soil chemical and microbial properties and endophyte profiles. This large dataset could be used to formulate hypotheses for future testing.

Objectives

1. To obtain a large dataset for establishing statistical association between canker expression, soil chemical and microbial properties and endophyte profiles

Material and methods

We decided to use the following scion cultivars: Golden Delicious, Grenadier, Gala, Braeburn, Jazz, Kanzi, and Rubens. Of these scion cultivars, Golden Delicious and Grenadier are resistant or tolerant to canker, whilst the others are all susceptible. Trees were grafted to M9 337 rootstocks.

Trees will be planted on two planting dates at three sites: Hononton Farm, Friday St. Farm, and Sheerland Farm. The first half of the trees will be planted before the end of 2018 and the second in the period of late Feb to early March 2019. On each plating date, there are 40 trees of each cultivar at each site; there are 1680 trees in total.

In November 2018 (at leaf fall) whilst still growing at the nursery, all trees will be sprayed with a moderate level of *N. ditissima* conidial suspension. This inoculation was used to ensure presence of a certain level of latent canker on all sites, increasing the usefulness of the data set to be collected.

We propose to sample bulk soil, roots and 1-year-old wood 6, 12 and 18 months after planting to characterise soil chemical properties, and profile soil microbiota and plant endophytes as well as plant hormones. These data will be analysed with several multivariate methods (e.g. canonical correspondence analysis, multivariate ANOVA, and redundancy analysis) to establish whether canker development is related to planting dates, specific soils, plant hormones, specific endophytes as identified in WP1, host genotypes, specific soil microbes or planting date. Furthermore, the data will reveal the extent to which the abundance and overall endophyte composition is influenced by factors other than host genotype.

Knowledge and Technology Transfer

(1) November 2017: Xiangming Xu presented the overview of the project at the third international apple canker and replant disease workshop held at NIAB EMR

(2) November 2017: Louisa Robinson-Bower presented the overview of using beneficial organisms to improve tree health at the third international apple canker and replant disease workshop held at NIAB EMR at NIAB EMR

(3) February 2018: Xiangming Xu presented the overview of the project at the AHDB agronomist day international apple canker and replant disease at NIAB EMR

References

Cota, L. V *et al*. (2008). Biological control of strawberry gray mold by Clonostachys rosea under field conditions. doi:10.1016/j.biocontrol.2008.04.023.

De Cal, A. *et al*. (2009). Population dynamics of Epicoccum nigrum, a biocontrol agent against brown rot in stone fruit. *J. Appl. Microbiol.* 106, 592–605. doi:10.1111/j.1365-2672.2008.04030.x.

Hashem, M. and Ali, E. (2004). *Epicoccum Nigrum* as biocontrol agent of *Pythium* damping-off and root-rot of cotton seedlings. *Arch. Phytopathol. Plant Prot.* 37, 283–297. doi:10.1080/03235400310001612955.

Larena, I. *et al*. (2004). Solid substrate production of Epicoccum nigrum conidia for biological control of brown rot on stone fruits. *Int. J. Food Microbiol.* 94, 161–167. doi:10.1016/j.ijfoodmicro.2004.01.007.

Ogórek, R. and Plaskowska, E. (2011). Epicoccum nigrum for biocontrol agents in vitro of plant fungal pathogens. *Commun. Agric. Appl. Biol. Sci.* 76, 691–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22702188 [Accessed October 21, 2018].

Arnold, A.E., *et al*. (2003). Fungal endophytes limit pathogen damage in a tropical tree. *PNAS,* 100: 15649-54.

Rodriguez, R.J., *et al*. (2008). Symbiotic regulation of plant growth, development and reproduction. *ISME J* 2, 404-416

Agler, M.T., *et al*. (2016). Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biology* 14, e1002352.

Wearn, J.A., *et al*. (2012). Species and organ specificity of fungal endophytes in herbaceous grassland plants. *Journal of Ecology* 100, 1085-1092.

Liu, Q., *et al*. 2011. Competition between foliar *Neotyphodium lolii* endophytes and mycorrhizal *Glomus* spp. fungi in *Lolium perenne* depends on resource supply and host carbohydrate content. *Functional Ecology* 25, 910-920.