Grower Summary

Systemic infection and symptom expression of Neonectria ditissima in relation to endophytes conditioned by environmental stresses

TF 226

Final report 2022

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Location of project:	East Malling, and several farms in Kent
Industry Representative:	Peter Checkley
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Most of 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.

GROWER SUMMARY

Headline

- One fungal endophyte showed good biocontrol potential against infection of leaf scars and pruning wounds by the canker pathogen.
- Cultivar responses to the canker pathogen is consistent across three sites, although the overall canker severity differed greatly among locations
- Between-season dynamics of endophytes in the leaf scars of new extension shoots suggest that exploitation of bacterial biocontrol agents could be more advantageous over the fungal ones since bacteria appeared to be more persistent between seasons
- Specific endophytes are associated with cultivar tolerance/resistance against the canker pathogen, but not the overall endophyte community
- The abundance of some endophytes is partially genetically controlled by the apple hosts; some genetic factors controlling endophytes are co-located or close to those controlling resistance against the canker pathogen
- Post-planting drought led to reduced tree development but did not affect canker development.
- Amendment with beneficial microbes at planting did not result in significant reduction in canker development.
- Planting soon after lifting led to reduced peripheral canker development, albeit a very limited reduction.

Background and expected deliverables

European canker

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.

Endophytes

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 specific endophytes.

Antagonist fungal endophytes and tolerance to N.ditissima

Recently, we have obtained preliminary data showing a link of antagonist fungal endophytes with cultivar tolerance to *N. ditissima*. 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*. In addition, we aim to understand the dynamics of an specific biocontrol endophyte colonising leaf scar tissues, to optimise their application strategy. 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. We are conducting experiments to assess (1) to what extent recruitment of endophytes is influenced by site-specific characteristics and host genotypes, and (2) whether canker symptom expression is related to planting times or the abundance of specific endophytes across several orchards. Finally, to assist in canker management, we are investigating the extent to which endophyte profiles of a specific apple genotype can be influenced by management practices (irrigation and soil amendment).

Summary of key project conclusions

We have successfully initiated all experimental studies on time; however, much of the lab molecular work has been delayed by at least six months because of COVID-19. We have applied for 6 month no-cost extension and are waiting for response from BBSRC.

- We have profiled endophytes at leaf scars of eight cultivars with differing tolerance/resistance against apple canker:
  a. Endophyte diversity was primarily affected by sampling time, orchard location, followed by location within an orchard and the scion, whereas the effect of rootstock was small.
  b. Several fungal and bacterial groups had differential relative abundance between canker resistant (tolerant) and susceptible cultivars. The specific fungal groups included fungal antagonists as well as plant pathogens.

c. Bacterial endophytes at the leaf scar tissues appear to be more consistent over two seasons, suggesting that exploitation of bacterial biocontrol agents could be advantageous over the fungal ones.

- One *Eppicocum* endophyte from apple has been shown to have good antagonistic effects against the apple canker pathogen in field tests:
  a. Co-inoculation of both Eppicocum and canker inoculum at leaf scars can reduce the canker incidence at leaf scar by 50%.
  b. For pruning cuts, there is very limited effects of *Eppicocum* probably because of greater susceptibility of fresh pruning cuts combined with a high dose of pathogen inoculum applied.
  c. Consistent with the endophyte dynamics, there is only a limited survival of this *Eppicocum* strain in the leaf scars over the winter
- Studying endophytes in a F_1 mapping population indicated
  a. Abundance of several endophytes is correlated with canker development.
  b. Abundance of specific endophytes is partially genetically controlled by the hosts.
  c. QTL mapping showed that a few QTL mapped for controlling endophytes are close to or co-locate with QTLs for canker resistance.
- Amending soils with PGPR (plant growth-promoting rhizobacteria) or AMF at the planting time has negligible effects on canker development.
- Post-planting drought led to reduced tree development but did not affect canker development.
- Testing seven scion cultivars at three orchards showed that:
  a. Longer duration of trees in cold storage initially led to increased canker incidence post-planting but two years after planting such an effect of cold-storage time on canker severity is negligible.
  b. Planting soon after lifting led to reduced peripheral canker development, albeit a very limited reduction.
  c. Cultivar susceptibility to the canker pathogen is consistent across the three orchards studied.
  d. There is some indication that symptom development of main stem cankers is affected by site specific factors; these cankers result most likely from nursery infections.

Financial benefits

This project is primarily a strategic research study. The only result that directly impacts commercial apple production most is the effect of storage duration on canker development, and also the effect of drought on tree development.

Action points for growers

- Plant trees as soon as possible after lifting.
- Ensure good uniform tree-establishment is important to reduce symptom development of main stem cankers, particularly those highly susceptible cultivars
- Avoid drought following planting