BS23288 *Neonectria* symptoms survey in the North Island Report Summary (November 2023)

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This paper summarises a project aimed to gain a wider understanding of the symptoms caused by one of the main kiwifruit trunk disease (KTD) pathogens, Neonectria microconidia, in the North Island and whether confirmed Neonectria symptoms with can be distinguished from other Neonectria-like KTD symptoms.

The study included a visual survey of 12 orchards in the North Island and collected valuable data on the fungal species complexes that are associated with KTD in New Zealand and the frequency at which they are found within the orchards surveyed. The results indicate that a range of fungal organisms are associated with KTD and was unable to distinguish Neonectria microconidia-specific symptoms from other KTD symptoms for diagnostics purposes in the sampled Gold3 vines.

The project report has been summarized below to remove confidential information, however please contact Zespri Innovation if you would like further information about this project.

Background

Trunk disease is broadly used to refer to various abnormalities and disease of woody perennial plants and is generally caused by various fungi that create damage and block the vascular system (Cloete et al. 2011). A range of plant pathogenic fungi are associated with kiwifruit trunk disease (KTD), which is beginning to represent a serious impediment to kiwifruit production.

Symptoms of KTD include a variety of external and internal wood decay symptoms including swollen trunks, cracking, cankers or discrete bulges of the trunk or leader, collar rot/crown decay, thinning canopies and the sudden death of a leader or the entire vine.

Although a number of fungi have been associated with KTD in New Zealand, few or none of these cause symptoms that can be solely attributed to them. One of the KTD organisms, *Neonectria microconidia*, is a relatively newly identified pathogen of kiwifruit vines in New Zealand (MPI 2016). *Neonectria microconidia* (also known as *Neonectria* canker of kiwifruit) has been isolated from kiwifruit vines with woody cankers of the leader and trunk, swellings, splitting and dieback of leaders. However, these are all symptoms that have also had other members of the KTD complex associated with them.

Methods

In total, 12 orchards (8 in Kerikeri, 1 in Whangarei and 3 in Te Puke) were surveyed by Verified Lab Services (VLS) for kiwifruit vine symptoms that could be attributed to *Neonectria microconidia* or

other KTDs in either the trunk or leader.

Five symptomatic Gold3 vines were marked from each orchard; photographs were taken of the disease symptoms and of the whole vine. Identifying data were recorded for each vine, including location, symptoms and whether there was visible dieback. Woody core samples were taken through symptomatic tissue using a clean and sterile 5-mm diameter Haglof forestry borer. The tissue was first thoroughly sprayed with ethanol. The core samples were placed into sterile labelled Falcon tubes using sterile forceps, then immediately held on ice. Samples were then transferred to the Mt Albert laboratory at The New Zealand Institute for Plant and Food Research Limited (PFR) within 24 h of collection. Sampling wounds were post- protected with pruning paint or similar to prevent further infection.

The five samples from each orchard underwent isolation and morphological identification. Alongside classical morphological diagnostics, two samples from each orchard also underwent DNA metabarcoding where ITS primers (Martin & Rygiewicz 2005) were used to amplify the fungal ribosomal ITS1 spacer region, spanning from the ribosomal 18S to 5.8S genes. Taxonomic identification of the Operational Taxonomic Units (OTU) sequences was performed using a BLASTN search of the ITS1 spacer region of the OTU sequences against the NCBI nt database. The results were filtered to remove unclassified hits and to amend the taxonomic assignment so that species was assigned if the hit had a percent identity (PI) of >99%. A genus was assigned if the hit had a PI of >97%.

Results

The most common KTD-associated fungi that were isolated from the 12 orchards (present in 10% or more of the vines) were the *Fusarium solani* species complex, *Ilyonectria spp., Neonectria microconidia, Cadophora dextrinospora* and *Neobulgaria alba* (see Table 1 below). Examples of the vines sampled and the organisms isolated from them are detailed in Appendix 1.

Table 1 Fungal isolations: Fungi known to be associated with kiwifruit trunk disease (KTD), the number of orchards from which they were isolated and the % of overall vines from which they were isolated.

Species	No. orchards (n=12)	% vines (n=60)
Fusarium solani species complex	12	51.7
Ilyonectria sp. (*Cylindrocarpon* sp.)	10	36.7
Neonectria microconidia (*Cylindrocarpon* sp.)	8	18.3
Cadophora dextrinospora	8	20.0
Neobulgaria alba	4	10.0
Basidiomycetes (all)	4	8.3

Discussion

The *Fusarium solani* species complex was the most common species isolated, being found in all 12 orchards and from 51.7% of the 60 vines sampled. Species of *Ilyonectria sp.* were found in 10 of the orchards and 36.7% of the vines, and *Neonectria microconidia* was identified from eight orchards, and from 18.3% of the vines overall. There appeared to be little difference in external symptomology that could distinguish between those from which the *Fusarium solani* species

complex, *Ilyonectria* spp. or *N. microconidia* were isolated. All three fungal groups were present in some vines and very few vines were solely colonised by a single KTD-associated organism.

Red spores (i.e. red perithecia) are a common characteristic with *Neonectria sp* infections. However, in this survey, only one of the six vines with 'red spores' (red perithecia) recorded had *N. microconidia*; while the others were *Fusarium solani* or *Ilyonectria* sp. This suggests that the presence of red perithecia is not a useful distinguishing characteristic to diagnose vines with *N. microconidia* (although it is useful for vines with KTD as a whole).

The DNA metabarcoding results were consistent with both the fungal isolations and with the species that we would expect to detect from trunk disease samples. These results give us confidence in the outcome of the work.

Metabarcoding appeared to detect a few extra organisms when compared with morphological diagnostics via fungal isolations, and some of these species may be more difficult to isolate into culture. For example, basidiomycetes are more difficult to isolate than ascomycetes; a survey of grapevine trunks in New Zealand resulted in few isolated basidiomycetes, despite specifically targeting them (Mundy et al. 2020).

In contrast to the metabarcoding, the isolation work was focused on the recovery of *Neonectria*, known members of the KTD complex, and fungi that are seen to be prevalent in each core. Fungi that were very uncommon were clearly not species associated with the symptoms of interest, and were therefore of low priority for further diagnostic examination.

While the metabarcoding work has proven useful in confirming the results of the isolation study, and has given some leads on more elusive fungi to target in the future (, it is probably not a practical or cost-effective technique for large-scale studies of KTD.

There was little difference in symptomology that could distinguish between the symptomatic vines from which the *Fusarium solani* species complex, *Ilyonectria* spp. or *N. microconidia* were isolated. All three fungal groups were present in some vines. Although this project was unable to distinguish KTD symptoms that could be used to diagnose *Neonectria microconidia* in Gold3 vines in Kerikeri, Whangarei or Te Puke, it has collected data on the fungal complex associated with KTD in New Zealand and the frequency at which they are found. These fungi are likely to spread through the vine slowly, possibly over several years, blocking the vascular system and eventually causing vine death. Directions for future research on the disease and fungal complex should include species distribution, epidemiology and control methods, as well as identifying which organisms are the primary pathogens.

References

Cloete M, Fourie PH, Damm U, Crous PW, Mostert L 2011. Fungi associated with die-back symptoms of apple and pear trees, a possible inoculum source of grapevine trunk disease pathogens.Phytopathol Mediterr 50: S176-S190.

Mundy DC, Brown A, Jacobo F, Tennakoon K, Woolley RH, Vanga B, Tyson J, Johnston P, Ridgway HJ, Bulman S 2020. Pathogenic fungi isolated in association with grapevine trunk diseases in New Zealand. N Z J Crop Hortic Sci 48(2): 84-96.

Appendix 1: Examples of the vines sampled and the organisms isolated from them

Vines where *Fusarium Solani* was isolated:		
		
		

Vines where *Ilyonectria* was isolated

Vines where *Neonectria microconidia* was isolated

Vines where *Cadosphora* was isolated

Vines where *Neobulgaria alba* was isolated

Vines where *Basidiomycete* was isolated

Vines where multiple KTD organisms were isolated from one sample