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BS19004 Nectriaceae associated with vine decline of kiwifruit. Objective 1. Incidence and prevalence

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Executive summary

BS19004 Nectriaceae associated with vine decline of kiwifruit. Objective 1. Incidence and prevalence

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Kiwifruit vine decline was first reported in New Zealand in c. 2000 and appeared to be associated with *Actinidia chinensis* var. *deliciosa* 'Hayward' vines over 15 years of age. At that time there were three main symptoms of vines: swellings below the main leader, uniformly enlarged trunks, and crown swelling. All symptom types showed evidence of decay within the wood and were therefore collectively referred to as **vine decay.**

With the incursion of *Pseudomonas syringae* pv. *actinidiae* (Psa) into New Zealand in 2010, research focus shifted away from kiwifruit vine decline and decay for many years. During that time the kiwifruit recovery plan was focussed on the removal of *A. chinensis* var. *chinensis* 'Hort16A' and re-grafting with new cultivars of unknown susceptibility to kiwifruit trunk diseases, e.g. Gold3 (*A. chinensis* var. *chinensis* 'Zesy002'), Green14 (*A. chinensis* var. *chinensis x A. chinensis* var. *deliciosa* 'Zesh004') and later, Red19 (*A. chinensis* var. *chinensis* 'Zes008'). In many orchards, these new cultivars are also reliant on aging rootstocks.

Within the recorded fungal pathogens that have been associated with root rot and trunk diseases of kiwifruit, there is a growing list of members of the Nectriaceae. Several species of the *Fusarium* and *Cylindrocarpon* complexes have been isolated from kiwifruit expressing general decline. Unpublished reports and records from New Zealand hint that there is quite a diverse set of species within the Nectriaceae that may be associated with this syndrome.

This project aimed to understand the incidence and prevalence of members of the fungal group Nectriaceae that are associated with kiwifruit vine decline. A survey of three kiwifruit orchards (two in the Bay of Plenty, one in Motueka), with a history of disease, took place in early 2019. In each orchard, fungal isolations were made from bark at the crown and from woody trunk cores taken from symptomatic and asymptomatic vines.

At the three orchards, the incidence of visibly diseased vines in the chosen block ranged from 18–34%. Fifteen species, types, or complexes within the Nectriaceae were identified from woody tissues of kiwifruit trunks and leaders, and from bark at the crown. Different principal fungal species/groups were identified in each orchard.

In Orchard 1 the *Ilyonectria* species group was most prevalent in the diseased vines (largely *Ilyonectria robusta*), and was recovered from 14.8% of all isolations; in the bark samples from diseased vines it made up 33%. Although not a member of the Nectriaceae, it was noticed that the fungus *Neobulgaria alba* (previously associated with kiwifruit swollen trunk syndrome and vine decline), was also found in the diseased trunks from Orchard 1. During a previous project in the

neighbouring block, *N. alba* was recovered from 32.4% of isolations from trunks with discoloured wood.

In Orchard 2, the *Fusarium solani* complex was most prevalent, making up 28.7% of all isolations from the diseased trunks. It was recovered from 34% of bark isolations and 41% of the 30 cm woody core isolations from the diseased vines.

In Orchard 3, *Neonectria microconidia* was recovered from 19.5% of all isolates from the diseased vines. It made up 65% of the isolations made from the centre of cankers on the leaders. Of the 10 cankers sampled, only one did not have *N. microconidia* present. The *Ilyonectria* group was also found at higher rates in the diseased vines in this orchard, making up 12.7% of all isolations. In contrast to Orchard 1, *Ilyonectria liriodendri* was the most common species from the *Ilyonectria* species group.

With a sample size of only three orchards, we are unable to draw any conclusions on the principal factors involved in the patterns seen. There are likely to be roles played by cultivar, rootstock age, region, environment, and previous history of each orchard block.

This project has provided a wealth of data that illustrates the complexity of vine decline/decay in kiwifruit. It also demonstrates how little is known about this syndrome/disease and the principal factors (biological and environmental) involved.

Recommendations for Objective 2 (pathogenicity testing)

Although many members of the Nectriaceae group were isolated from declining/decaying kiwifruit vines, this does not necessarily mean that these are causing the various symptoms observed. Some species may be the primary cause of decline, others may be part of a complex, and others may be just taking advantage of declining vines and the presence of other fungi.

Objective 2 of this project is the verification of pathogenicity of different Nectriaceae isolates on kiwifruit cultivars. Four species or groups were isolated most frequently — the *F. solani* complex, *N. microconidia*, the *Ilyonectria* species group and *Clonostachys* sp. Of these, *N. microconidia* and the *Ilyonectria* species group are the subject of pathogenicity testing in other projects (BS19011 and BS1932), using isolates found in this study, therefore this does not need to be repeated here. *Clonostachys* sp. is likely to be a saprophyte on dead or dying plant tissues and is not a good candidate for pathogenicity testing.

Sequencing results of members of the *F. solani* complex isolated in this work have shown that there is an array of types/strains of *F. solani* found in the wood of kiwifruit vines, particularly those that are visibly diseased. *F. solani* is a known plant pathogen and it is suggested that a variety of these strains are included in pathogenicity testing. Although the other five *Fusarium* spp. identified were at low numbers, *Fusarium cerealis* was found regularly and may be a candidate for testing. *Fusarium venenatum* was found at even lower rates but was the major species found in one diseased vine and could also be useful to test.

Recommended groups for pathogenicity testing in Objective 2:

- *Fusarium solani* complex
- *Fusarium cerealis*
- *Fusarium venenatum*

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1 Programme overview

1. Understanding the incidence and prevalence of members of the Nectriaceae associated with kiwifruit vine decline.
2. Verification of the pathogenicity of different isolates on kiwifruit cultivars
3. A literature review to identify potential treatments to support extension messages that may inform management decisions by growers.

This work comprises Objective 1 of a wider programme of research. It involves a survey of three kiwifruit orchards (two in the Bay of Plenty, one in Motueka), with a history of disease, to establish rates of infection by members of the Nectriaceae and a culture collection. This culture collection will subsequently be used in assays to determine pathogenicity (Objective 2). Lastly, a literature review will be completed to try and identify potential treatments and methods that may support extension messages to growers. An outcome of this research would include better information to help plan future research and management priorities for target organisms.

This project is closely linked to projects BS1932 (Identification and characterisation of *Ilyonectria* species associated with root rot of kiwifruit) and BS19011 (*Neonectria* pathogenicity). A previous project, BS20117, also focussed on fungal isolations of vine decay symptoms in one of the orchards surveyed in this project (Tyson et al. 2020).

2 Introduction

Unlike grapevine trunk diseases (GTD), which have been studied for well over a century (Gramaje et al. 2018), there has been relatively little research on vine decline/decay of kiwifruit.

Vine decline was first reported in New Zealand kiwifruit in c. 2000 (Manning et al. 2002). A grower survey at that time showed that the disorder was associated with *Actinidia chinensis* var. *deliciosa* 'Hayward' vines over 15 years of age. Manning et al. (2002) found that there were three main symptoms of vines: swellings below the main leader, uniformly enlarged trunks, and crown swelling. All symptom types showed evidence of decay within the wood and were therefore collectively referred to as **vine decay**. This early work by Manning et al. (2002) was preliminary, but did establish an association between the fungus *Phialophora alba* and symptomatic vines. *P. alba* was subsequently re-named *Neobulgaria alba* by Johnston et al. (2010).

Later, Manning et al. (2007) re-classified the symptoms in 'Hayward' as **swollen trunk disorder** (commonly associated with *N. alba*) and **crown decay disorder** (inconclusive causal agents, but some association with *Phytophthora*, *Pythium* and other root pathogens). At this time, Manning et al. (2007) also documented vascular disease in *Actinidia chinensis* var. *chinensis* 'Hort16A' for the first time in New Zealand, classifying symptoms into four types: (1) rootstock disorder commonly associated with *Cylindrocarpon* species, (2) leader die-back commonly associated with *Cylindrocarpon* species, (3) spongy bark disorder commonly associated with *Verticillium* species and (4) crown gall-like disorder in nursery vines. At a similar time, 'Hort16A' kiwifruit vines in Italy were found to be susceptible to vascular diseases (Riccioni et al. 2007).

With the incursion of *Pseudomonas syringae* pv. *actinidiae* (Psa) into New Zealand in 2010, research focus shifted away from kiwifruit vine decline and decay for many years. During that time the kiwifruit recovery plan was focussed on the removal of 'Hort16A' and re-grafting with new cultivars of unknown susceptibility to kiwifruit trunk diseases, e.g. Gold3 (*Actinidia chinensis* var. *chinensis* 'Zesy002'), Green14 (*Actinidia chinensis* var. *chinensis x A. chinensis* var. *deliciosa* 'Zesh004') and, later, Red19 (*Actinidia chinensis* var. *chinensis* 'Zes008').. In many orchards, these new cultivars are also reliant on aging rootstocks.

A suite of fungi have been recovered in association with kiwifruit vine decline/decay or kiwifruit elephantiasis overseas, such as *Cadophora luteo-olivacea*, *C. malorum*, *C. melinii*, *Lecythophora luteoviridis*, *Phaeoacremonium aleophilum*, *Ph. mortoniae* and *Ph. parasiticum*; and *Fomitiporia punctata* (Di Marco et al. 2000; Di Marco et al. 2003; Di Marco et al. 2004; Prodi et al. 2008). *Botryosphaeria* spp. and *B. dothidea* have also been reported causing wood decay and dieback, respectively, of kiwifruit in Italy and Greece (Elena and Paplomatas 2002; Abbatecola et al. 2008; Thomidis and Exadaktylou 2010).

Armillaria novae-zelandiae has long been known to cause root rot and death of kiwifruit vines in New Zealand, particularly after felling of shelter-belt trees (Horner 1992). In addition, many *Phytophthora* and *Pythium* species have been recovered from kiwifruit in different countries, including New Zealand (Stewart and McCarrison 1991; Erwin and Ribeiro 1996; Donati et al. 2020).

Within the recorded fungal pathogens that have been associated with root rot and trunk diseases of kiwifruit, there is a growing list of members of the Nectriaceae. Several species of the *Fusarium* and *Cylindrocarpon* complexes have been isolated from kiwifruit expressing general decline (Table 1). The New Zealand "grey literature" (unpublished reports and records) hint that there is quite a diverse set of species within the Nectriaceae that are associated with this syndrome (Table 2).

Table 1. Published records of members of the Nectriaceae associated with root and trunk diseases of *Actinidia* spp. worldwide.

Species	Symptom	Country	Reference
Cylindrocladiella parva		Turkey	(Erper et al. 2013)
Dactylonectria pauciseptata (as *Cylindrocarpon pauciseptatum*)		Turkey	(Erper et al. 2013)
Ilyonectria europaea		Turkey	(Erper et al. 2013)
Ilyonectria liriodendri		Turkey	(Erper et al. 2011)
Ilyonectria robusta		Turkey	(Erper et al. 2013)
Ilyonectria torresensis		Turkey	(Erper et al. 2013)
Cylindrocladium crotalaria (Calonectria crotalaria)	Root rot	USA, South Carolina	(Krausz and Caldwell 1987)
Cylindrocarpon spp.	Trunks, roots	Italy	(Nipoti et al. 2003)
Fusarium spp.	Trunks, roots	Italy	(Nipoti et al. 2003)

Table 2. New Zealand "grey literature" (unpublished) records of members of the Nectriaceae associated with root and trunk diseases of *Actinidia* spp.

Fungal species	Region	Reference
Cylindrocarpon spp.	Bay of Plenty	(Manning et al. 2009)
Dactylonectria pauciseptata (syn. *Cylindrocarpon pauciseptatum*)	Bay of Plenty	(MPI 2018a, b)
Dactylonectria sp.	Bay of Plenty	(MPI 2018b)
Fusarium culmorum	Bay of Plenty	(MPI 2017)
Fusarium equiseti	Bay of Plenty	(MPI 2018b)
Fusarium oxysporum	Bay of Plenty	(Manning et al. 2009)
Fusarium solani	Bay of Plenty, Kerikeri	(MPI 2017, 2018b), Tyson unpub.
Fusarium sp.	Bay of Plenty, Kerikeri	Tyson unpub.
Ilyonectria liriodendri (as *Cylindrocarpon lireodendri*)	Bay of Plenty	(Manning et al. 2009)
Ilyonectria robusta	Bay of Plenty	(MPI 2018b)
Ilyonectria sp.	Bay of Plenty, Kerikeri	(MPI 2017, 2018b) (Tyson et al. 2020)
Ilyonectria sp. (as *Cylindrocarpon* sp.)	Bay of Plenty, Kerikeri	Tyson unpub.
Neonectria microconidia	Bay of Plenty	(MPI 2018a), (Tyson et al. 2019; Tyson and Mellow 2020; Tyson et al. 2020)
Thelonectria sp.	Bay of Plenty	(MPI 2018b)

This project aims to understand the incidence and prevalence of members of the fungal group Nectriaceae associated with kiwifruit vine decline and involves a survey of three kiwifruit orchards with a history of disease (two in the Bay of Plenty, one in Motueka). This report details the fungal isolations, subsequent identifications and rates of infection by members of the Nectriaceae in woody trunk cores taken from symptomatic and asymptomatic vines, in order to identify the possible association of these micro-organisms with vine symptoms.

3 Methods

3.1 Survey sites

Three orchards with vine decline, canker or trunk decay symptoms were identified by Kiwifruit Vine Health (KVH) in early 2019 (Table 3). Previously we have found that vine decline symptoms are expressed more strongly after spring growth has been completed and the vines are beginning to come under stress, potentially from fruit load or drier conditions. Therefore, orchards were visited in March/April 2019. In each orchard, a single kiwifruit block was mapped and the symptoms seen on each vine recorded. For sampling, 10 symptomatic and 10 mostly asymptomatic kiwifruit vines were identified from each of the three blocks.

Orchard 1 was located in Paengaroa, Bay of Plenty. In the chosen block, the older vines were 'Hayward' on ‘Bruno’ rootstock, while younger vines were 'Hayward' on ‘Kaimai’ rootstock. All of the sampled vines were the older ‘Hayward’ on ‘Bruno’ rootstock. There were no signs of cankers in the canopy, and most symptomatic vines had collar rot.

Orchard 2 was located in Te Puke, Bay of Plenty. The chosen block was Gold3 on ‘Bruno’ rootstock. The rootstock is thought to be c. 30 years old, and was grafted to Gold3 in c. 2011. The grafts were very low on the vines; c. 30-40 cm above the soil. There was no sign of cankers in the canopy, and most symptomatic vines had collar rot up to the graft site.

Orchard 3 was located in Motueka, South Island. The vines were Gold3 on an unknown rootstock. This block had obvious, often extensive cankers in the canopy (cracking, bulging of the leader/scion). Many of the symptomatic vines also had collar rot.

Table 3. Details of kiwifruit orchards surveyed for vine decline symptoms.

Orchard	Orchard	KPIN	Cultivar	Sample date
1	[redacted]	[redacted]	‘Hayward’ [a]	4–5 March 2019
2			Gold3 [b]	1–2 April 2019
3			Gold3	8–9 April 2019

[a] Actinidia chinensis var. deliciosa ‘Hayward’
[b] Actinidia chinensis var. chinensis ‘Zesy002.’

3.2 Sample collection

Samples were taken from at least three locations on each vine. Bark samples were taken from the base (crown) of each vine with a sharp, sterilised knife. Transverse woody core samples were taken at 30 cm and 100 cm above the soil with sterile 5-mm diameter forestry corers. Prior to taking cores, the areas were thoroughly sprayed with 90% ethanol. Additional samples were taken if cankers were present. Between each sample, the corers were cleaned and washed with 90% ethanol to prevent

cross-contamination. After taking samples, the wounds were sprayed with copper fungicide (Nordox™ 75G) and then sealed with Bacseal Super pruning paint to prevent re-infection. Samples were transferred to the laboratory in sterile Falcon tubes within 24 h of collection.

3.3 Fungal isolation

Each entire core sample was surface sterilised (30 s in 70% ethanol, 5 min in 0.35% sodium hypochlorite, 30 s in 70% ethanol and a wash in sterile distilled water). The cores were then cut into 1 cm segments, placed on Difco potato dextrose agar (PDA) amended with rifampicin and streptomycin to prevent bacterial growth. Plates were incubated at c. 22°C (room temperature) under natural light.

Sub-cultures were made of fungi that emerged from the core sections, focussing on the recovery of members of the *Cylindrocarpon* complex and *Fusarium* species (Nectriaceae). For initial identifications, standard morphological techniques (e.g. colony characteristics, presence and morphology of reproductive structures) were used to identify the resulting isolates to family and genera, targeting members of the Nectriaceae.

3.4 DNA sequencing

DNA was extracted from mycelia of representative isolates using a QIAGEN DNeasy® Plant Mini Kit, following the manufacturer's instructions.

The desired gene regions of each isolate were amplified by Polymerase chain reaction (PCR) with one or more of the chosen primer pairs (Table 4). PCR products were visualised on a 1.5% agarose gel and then purified using a QIAGEN QIAquick PCR Purification Kit, following manufacturer's instructions, and sequenced at MacroGen (Seoul, South Korea).

The most likely taxonomic identity of each fungal isolate was determined using the Geneious BLASTn function to compare the sequences generated in this study with those stored in a nucleotide collection database (GenBank).

Some of the *Ilyonectria* group isolates collected in this study were sequenced as part of project BS1932 (Ridgway and Tyson 2018; Ridgway et al. 2019). Others were identified using the primer pair CYLH3F - CYLH3R.

Table 4. Primer pairs used for identification of Nectriaceae species.

Primer pair	Primer sequence 5'-3'	Gene	Reference
CYLH3F CYLH3R	AGGTCCACTGGTGGCAAG AGCTGGATGTCCTTGGACTG	Histone	Crous et al. (2004)
EF1aF EF1aR	CATCGAGAAGTTCGAGAAGG TACTTGAAGGAACCCTTACC	translation elongation factor 1 alpha	Carbone and Kohn (1999)
ITS4 ITS5	TCCTCCGCTTATTGATATGC GGAAGTAAAAGTCGTAACAAGG	internal transcribed spacer	White et al. (1990)
BT2A BT2B	GGTAACCAAATCGGTGCTGCTTTC ACCCTCAGTGTAGTGACCCTTGGC	beta tubulin	Glass and Donaldson (1995)

4 Results

4.1 Symptoms observed

At the three orchards, the incidence of visibly diseased vines ranged from 18–34% (Table 5). It is likely that some of the asymptomatic vines were also at an early stage of disease as the wood core samples of some had areas of discolouration. Maps of the orchard blocks, showing “healthy” and diseased vines and the locations of sampled vines, are given in Appendix 1 (Figure A1-1, Figure A1-2 and Figure A1-3). Young vines, presumably replacements for those removed due to disease, are noted separately.

Table 5. Incidence of “healthy” (asymptomatic), young and visibly diseased kiwifruit vines in each orchard.

	total # vines	'healthy'	young	diseased
Orchard 1 (Paengaroa)	494	54%	22%	24%
Orchard 2 (Te Puke)	414	79%	3%	18%
Orchard 3 (Motueka)	483	64%	2%	34%

Types of symptoms seen in the three orchards included collar rot, cankers, swelling and cracking of leaders, swollen trunks, rotting at or near the graft and sparse canopies (Figure 1). Each orchard had different symptoms that were most noticeable. Orchard 1 had high numbers of vines with collar rot and swollen trunks, Orchard 2 had high numbers of vines with collar rot and Orchard 3 was distinguished by the prevalence of obvious cankers, and swelling and cracking of leaders. It is likely that the swelling and cracking of leaders in Orchard 3 was a pre-cursor to cankers forming. Table 6 shows the incidence of each symptom type in each orchard (note that some vines displayed more than one symptom type).

Table 6. Incidence of each type of symptom in each orchard.

	# diseased vines	Collar rot	rot at/near graft	Swollen trunk	Swelling/ swelling of leader	Canker	Sparse canopy
Orchard 1	117	63%	8%	34%	0%	2%	12%
Orchard 2	73	79%	8%	19%	0%	0%	10%
Orchard 3	165	28%	10%	1%	47%	30%	4%

Figure 1. Severe collar rot and canker symptoms in Orchards 2 and 3.

4.2 Fungal identifications

Fifteen species, types, or complexes within the Nectriaceae were identified from kiwifruit trunks, leaders or bark (Table 7).

Table 7. Members of the Nectriaceae identified from woody tissues of kiwifruit vines in three New Zealand orchards.

Identification	Notes
Clonostachys sp.	syn. *Gliocladium* sp. One isolate was identified to species and was sequenced as *Clonostachys rosea*
Dactylonectria sp.	*Dactylonectria* species not resolved to species by sequencing
Fusarium avenaceum	-
Fusarium cerealis	syn. *F. crookwellense*
Fusarium equiseti	-
Fusarium oxysporum	-
Fusarium solani complex	includes *Fusarium striatum*
Fusarium sp.	Fusarium isolates from the red-pigmented group not identified to species (likely *F. avenaceum, F. cerealis, F. equiseti* or *F. venenatum*)
Fusarium venenatum	differentiated from *F. sambucinum* in 1995
Ilyonectria europaea	-
Ilyonectria liriodendri	syn. *Cylindrocarpon liriodendri*
Ilyonectria robusta	-
Ilyonectria sp.	*Ilyonectria* isolates not identified to species (likely I. europaea, I. liriodendri, *I. robusta* or *I. torresensis*)
Ilyonectria torresensis	≡ *Dactylonectria torresensis*
Mariannea sp.	*Mariannea* species not resolved to species by sequencing
Neonectria microconidia	Differentiated from the Neonectria coccinea group in 2011
Thelonectria sp.	*Thelonectria* species not resolved to species by sequencing

4.3 Orchard trends

The incidence of the different groups of fungi isolated from diseased and asymptomatic kiwifruit vines from the three orchards is shown in Table 8.

In Orchard 1, the *Ilyonectria* species group was most prevalent in the diseased vines, being recovered from 14.8% of all isolations; in the bark samples from diseased vines it made up 33% (Figure 2). In this orchard, *I. robusta* was the most common species from the *Ilyonectria* species group.

Although most species that were not of the Nectriaceae family were not identified, it was noticed that the fungus *Neobulgaria alba*, which has previously been associated with kiwifruit swollen trunk syndrome and vine decline (Manning et al. 2002; Johnston et al. 2010), was also found in the diseased trunks from Orchard 1. During a previous project (BS20117) in the neighbouring block, *N. alba* was recovered from 32.4% of isolations made at c. 1–1.2 m above the soil from trunks with discoloured wood. In that work it was not recovered from asymptomatic vines (Tyson et al. 2020).

In Orchard 2, the *F. solani* complex was most prevalent, making up 28.7% of all isolations from the diseased trunks. It was recovered from 34% of bark isolations and 41% of the 30 cm woody core isolations from the diseased vines.

In Orchard 3, *Neonectria microconidia* was recovered from 19.5% of all isolates from the diseased vines. It made up 65% of the isolations made from the centre of cankers on the leaders. Of the 10 cankers sampled, only one did not have *N. microconidia* present.

The *Ilyonectria* group was also found at higher rates in the diseased vines in this orchard, making up 12.7% of all isolations. In contrast to Orchard 1, *Ilyonectria liriodendri* was the most common species from the *Ilyonectria* species group.

Table 8. Incidence of different taxa isolated from diseased and asymptomatic kiwifruit vines from the three orchards. Fungal groups that had a higher incidence (<2% increase) in the symptomatic trunks than the "healthy" trunks are highlighted in orange.

	Orchard 1		Orchard 2		Orchard 3	
	healthy	diseased	healthy	diseased	healthy	diseased
Fusarium solani complex	2.6%	5.2%	7.2%	28.7%	2.2%	3.3%
Fusarium spp.	1.6%	2.3%	4.2%	3.5%	5.2%	7.0%
Neonectria microconidia	2.6%	1.0%	8.0%	4.7%	4.5%	19.5%
Ilyonectria spp.	4.5%	14.8%	3.8%	5.1%	6.7%	12.7%
Clonostachys sp.	1.0%	3.1%	11.4%	15.7%	19.9%	19.8%
Other Nectriaceae	0.0%	0.3%	0.4%	0.0%	0.4%	0.3%

The prevalence of each Nectriaceae species is shown as "heat maps" in Figure 2, Figure 3 and Figure 4. These show the rate at which each species was recovered from the sampled kiwifruit trunks at each point on the vine trunks; in bark from the crown, and in wood cores at 30 cm and 100 cm above the soil. Percentages are the proportion of isolations at each height from which each species was recovered. Ten "healthy" vines and 10 diseased vines were sampled at each orchard.

Orchard 1 (Paengaroa) 'Hayward'	"healthy" vines			diseased vines		
	bark	30 cm	100 cm	bark	30 cm	100 cm
Ilyonectria sp.	3%	1%		14%	1%	9%
Ilyonectria europaea		4%	3%		2%	1%
Ilyonectria liriodendri						
Ilyonectria robusta	5%			19%	3%	4%
Ilyonectria torresensis					2%	
Dactylonectria sp.						
Mariannea sp.						
Thelonectria sp.					1%	
Neonectria microconidia		4%	2%	1%	1%	1%
Fusarium solani complex		4%	2%	4%	5%	6%
Fusarium sp.						
Fusarium avenaceum						
Fusarium cerealis		3%		1%		
Fusarium equiseti				3%		
Fusarium venenatum	3%				4%	
Fusarium oxysporum						
Clonostachys sp.	3%	1%		11%	1%	1%

Figure 2. Orchard 1. Heat map of the prevalence of each species in sampled kiwifruit trunks; in bark from the crown, and in wood cores at 30 cm and 100 cm above the soil.

Orchard 2 (Te Puke) Gold3	"healthy" vines			diseased vines		
	bark	30 cm	100 cm	bark	30 cm	100 cm
Ilyonectria sp.	3%				4%	8%
Ilyonectria europaea		6%				
Ilyonectria liriodendri						
Ilyonectria robusta						3%
Ilyonectria torresensis						
Dactylonectria sp.						
Mariannea sp.			2%			
Thelonectria sp.						
Neonectria microconidia	5%	12%			8%	
Fusarium solani complex		12%		24%	41%	2%
Fusarium sp.	3%	1%	2%	10%		
Fusarium avenaceum						
Fusarium cerealis						3%
Fusarium equiseti		1%				
Fusarium venenatum		1%		5%		
Fusarium oxysporum	5%	2%				2%
Clonostachys sp.	28%	11%	2%	24%	20%	

Figure 3. Orchard 2. Heat map of the prevalence of each species in sampled kiwifruit trunks; in bark from the crown, and in wood cores at 30 cm and 100 cm above the soil.

Orchard 3 (Motueka)	"healthy" vines			diseased vines			
Gold3	bark	30 cm	100 cm	bark	30 cm	100 cm	canker
Ilyonectria sp.	2%	1%	3%	8%	4%	3%	
Ilyonectria europaea			1%	4%	1%	3%	
Ilyonectria liriodendri		4%	4%		8%	8%	4%
Ilyonectria robusta	2%		3%	10%			2%
Ilyonectria torresensis							
Dactylonectria sp.				2%			
Mariannea sp.							
Thelonectria sp.		1%					
Neonectria microconidia	7%	2%	7%	6%	4%	13%	65%
Fusarium solani complex	2%	2%	3%		5%	3%	2%
Fusarium sp.	2%	2%		2%	2%	1%	2%
Fusarium avenaceum		1%	4%	2%	1%		
Fusarium cerealis	4%	1%	1%	2%	6%		
Fusarium equiseti						4%	
Fusarium venenatum				4%			
Fusarium oxysporum		1%			1%		
Clonostachys sp.	15%	18%	29%	23%	28%	15%	6%

Figure 4. Orchard 3. Heat map of the prevalence of each species in sampled kiwifruit trunks; in bark from the crown, in wood cores at 30 cm and 100 cm above the soil, and from cankers on the leader (if present).

4.4 Individual vines

Within each orchard, the individual vines revealed many patterns of infection/colonisation. Maps of the results from each isolation on each individual vine are shown for the three orchards in Appendix 2 (Figure A2-1, Figure A2-2 Figure A2-3).

As seen in the heat maps (Section 4.3 above), different fungi were prevalent at each sampling site, for example, in the diseased vines from Orchard 2 the *F. solani* complex was the most common fungus recovered from the 30 cm woody cores, but was only recovered once from the cores taken from 1 m. In addition to this, different fungi were found at different parts of the core sample; for example, *F. solani* was more likely to be found in the centre of the cores, as was *N, microconidia*, whereas members of the *Ilyonectria* group were more likely to be found at either end of the core (the outermost area of the trunks).

Members of the *Ilyonectria* species group were found, sporadically, at all heights on the vines, and on all orchards. It is likely that the overall picture of this group is confused due to four species were found; none were common enough alone to give a clear picture. For example, *I. liriodendri* was only found in Orchard 3 (Motueka), and *I. torresensis* was only found in the 30 cm core of one vine in Orchard 1. In several vines, two species of *Ilyonectria* were present, and in one case, different species were found at either ends of one woody core sample (different sides of the trunk).

In Orchard 3, *Clonostachys* sp. (syn. *Gliocladium* sp.) was more prevalent than in Orchards 1 and 2, but was found at similar levels in the asymptomatic and the diseased vines. It also appeared most

often in tandem with other species, e.g. *N. microconidia*. *Clonostachys* species are best known as saprophytes, tend to grow faster than many other fungi in vitro, and grow over other fungal colonies, and so is likely to have also obscured other fungi within the isolation plates.

The other *Fusarium* spp. (*Fusarium avenaceum*, *F. cerealis*, *F. equiseti*, and *F. venenatum*) were identified were at low numbers. *F. cerealis* was found regularly, at all three orchards and at all sampled heights on the vines. *Fusarium venenatum*, while relatively rare, was the major species found in one diseased vine.

4.5 *Neonectria* cankers on Gold3 vines in Motueka

The unusual canker symptoms observed in Orchard 3 (Motueka) were studied in greater detail. The symptoms consisted of significant swelling, splitting and leader death. In some cases red perithecia (spore-forming structures) were observed and in many cases the cankers appeared to be associated with wounds.

Woody cores were taken from four vines with well-developed cankers. The cores were taken from five sections across the vine; from:

1. 20 cm below the visible lower edge of the canker
2. the lower edge of the canker
3. the centre of the canker
4. the upper edge of the canker,
5. 20 cm above the upper edge of the canker.

Fungal isolations were made from the cores, as described in Section 3.3.

Figure 5 shows where *N. microconidia* is present across and along each canker, from 20 cm below the lower edge (closest to the trunk) of the visible canker, to 20 cm above the upper edge. Isolations were made at 1 cm intervals across the leaders.

vine	20 cm below canker edge	lower edge of canker	centre of canker	upper edge of canker	20 cm above canker edge
14	other	Botryosphaeria, Clonostachys s	I. liriodendri	Neo. microconidia	Phomopsis, Clonostachys sp.
	other	Neo. microconidia	Neo. microconidia, I. liriodendri	Neo. microconidia	Phomopsis, Clonostachys sp.
	other	Neo. microconidia	Neo. microconidia, I. liriodendri	Neo. microconidia	Clonostachys sp.
	other	Neo. microconidia	F. solani	Neo. microconidia	Clonostachys sp.
	other	Neo. microconidia	Neo. microconidia	Neo. microconidia	Clonostachys sp.
	Mucor	Neo. microconidia	Neo. microconidia	Neo. microconidia	Clonostachys sp., Epicoccum
		Phomopsis, Clonostachys sp.		Clonostachys sp., Epicoccum	
15	Phomopsis	Phomopsis	Neo. microconidia	Phomopsis	Phomopsis
	Phomopsis, Clonostachys sp.	Neo. microconidia	Neo. microconidia	Neo. microconidia	other
	Clonostachys sp.	NG	Neo. microconidia	Neo. microconidia	other
	Clonostachys sp.	other	Neo. microconidia	other	other
	Neo. microconidia	other	Neo. microconidia	Phomopsis	other
	Neo. microconidia	Phomopsis	Neo. microconidia		other
			Neo. microconidia		
			Neo. microconidia, Phomopsis,	Clonostachys sp.	
16	F. cerealis	other	Neo. microconidia, Phomopsis	Phomopsis	other
	Clonostachys sp.	Clonostachys sp.	Neo. microconidia	Neo. microconidia	other
	other	other	Neo. microconidia	Neo. microconidia	other
	other	Clonostachys sp.	F. solani, I. robusta	Neo. microconidia	other
	Phomopsis	Neo. microconidia	Neo. microconidia, I. robusta	Neo. microconidia	Phomopsis
		Phomopsis	Neo. microconidia	Phomopsis	
			Phomopsis	Phomopsis	
			Neo. microconidia		
19	Phomopsis	Phomopsis	Clonostachys sp.	Phomopsis	NG
	Phomopsis	Neo. microconidia	Fusarium sp.	Phomopsis	NG
	other	Neo. microconidia	Neo. microconidia	Neo. microconidia	NG
	Neo. microconidia, other	Neo. microconidia	Neo. microconidia	Neo. microconidia	NG
	other	Neo. microconidia	Neo. microconidia	Neo. microconidia	other
	other, Clonostachys sp.	Neo. microconidia	Neo. microconidia	Phomopsis	
		Neo. microconidia	other		
			Neo. microconidia		
			Clonostachys sp.		

Figure 5. Cross-sections of cankers from four vines in Orchard 3 (Motueka), showing the locations at which *Neonectria microconidia* was isolated across and along the cankered leaders. NG = no fungal growth.

Although pathogenicity testing of *N. microconidia* has not yet been completed, it is clear that this species is highly associated with the cankers seen on Gold3 in Motueka. In the four cankers from which longitudinal and cross-section sampling was undertaken, *N. microconidia* was recovered from 74% of the isolations taken from the centre of the cankers, and from 50% and 60% of the isolations from the lower and upper edge of the cankers, respectively. In contrast, it was only found from 13% and 0% of the isolations made from 20 cm below and 20 cm above the cankers, respectively (Table 9).

Table 9. Proportions of isolations yielding *Neonectria microconidia* from each site along cankers from Gold3 (*Actinidia chinensis* var, *chinensis* 'Zesy002') kiwifruit vines in Motueka (data from four cankers).

Position	# isolations	# isolations *Neonectria microconidia*	% isolations *Neonectria microconidia*
20 cm below canker edge	23	3	13%
lower edge of canker	26	13	50%
centre of canker	31	23	74%
upper edge of canker	25	15	60%
20 cm above canker edge	22	0	0%

5 Discussion

This project has provided a wealth of data that illustrates the complexity of vine decline/decay in kiwifruit. It also demonstrates how little is known about this syndrome/disease in kiwifruit and the principal factors (biological and environmental) involved.

As with grapevine trunk disease (GTD) (Gramaje et al. 2018), the recent incidence, or perhaps increasing visibility/acknowledgement, of kiwifruit trunk diseases is likely the result of several factors. Firstly, the boom of kiwifruit planting in New Zealand from the 1980s onwards has resulted in a large cohort of aging vines/rootstocks and therefore an increase in orchard blocks reaching an age where symptoms are more obvious.

Secondly, the practice of re-grafting older vines to new cultivars, firstly to 'Hayward', then to 'Hort16A', and more recently (post-Psa) to the newer cultivars, has:

- created large trunk wounds that are exposed to inoculum at each new graft (even with protectants)
- grafted new cultivars onto potentially infected older vines
- allowed the discolouration within cut trunks to be seen.

Thirdly, changes in production methods that are likely to favour fungal infection have also occurred, for example "stump grafting" and the widespread use of 'girdling' of the trunks. Potentially we are also seeing the effect of kiwifruit orchards becoming established in more marginal areas, particularly those that are less well drained, or prone to flooding or frosts.

Nectriaceae identified

Fifteen species, types, or complexes within the Nectriaceae were identified from kiwifruit trunks, leaders or bark. Four species or groups were isolated most frequently - the *F. solani* complex, *N. microconidia*, the *Ilyonectria* species group and *Clonostachys* sp.

There were large differences in the Nectriaceae communities seen in each orchard, but also between and within individual vines within each orchard. Vines that were asymptomatic were frequently host to a large number of pathogens from the Nectriaceae, indicating that colonisation is occurring well before vines become visibly diseased.

Many of the cankers observed in Orchard 3 (Gold3, Motueka) appeared to be associated with pruning wounds. It was clear from the longitudinal sampling of cankered leaders that *N. microconidia* is highly associated with these cankers. This work also indicated that when pruning is undertaken to remove cankers, this needs to be performed well below the visible canker as the fungus may be present at least 20 cm below the lower edge of the canker. Interestingly, *N. microconidia* was present in all orchards within the trunks, both symptomatic and asymptomatic. It is currently unknown what triggers the formation of cankers in some situations; however, likely factors are environmental stresses (e.g. frost), cultivar and/or wounds.

Orchard hygiene

Although there appears to be a complex of fungi within the Nectriaceae involved in kiwifruit vine/decline and decay, the advice given by Manning and colleagues (Manning et al. 2002; Manning et al. 2010) is still important. They wrote that if pathogens associated with trunk disease

enter the vine through pruning wounds, then the incidence could be reduced by improved pruning practices. For example, destroying prunings to prevent dispersal and applying pruning paste to large wounds. In addition, sensible hygiene of girdling and pruning instruments was strongly recommended. When removing affected vines, their advice was to not replace vines in the same planting hole.

Many of the pathogens identified in the current work will likely also be in the soil and very difficult to eradicate.

Recommendations for Objective 2 (pathogenicity testing)

Fifteen species, types, or complexes within the Nectriaceae were identified from woody tissues of kiwifruit trunks and leaders, and from bark at the crown.

Although many members of the Nectriaceae group were isolated from declining/decaying kiwifruit vines, this does not necessarily mean that these are causing the various symptoms observed. Some species may be the primary cause of decline, others may be part of a complex, and others may be just taking advantage of declining vines and the presence of other fungi.

Objective 2 of this project is the verification of pathogenicity of different Nectriaceae isolates on kiwifruit cultivars. The species chosen will ultimately depend on a combination of the frequency of isolation, published history of each species as a plant pathogen and a comparison of the fungi found in symptomatic vines versus those found in asymptomatic vines.

Four species or groups were isolated most frequently: the *F. solani* complex, *N. microconidia*, the *Ilyonectria* species group and *Clonostachys* sp. The genera *Fusarium*, *Neonectria* and *Ilyonectria* are known to contain plant pathogenic species and may be involved in the kiwifruit vine decline/trunk decay that has been seen in the three orchards.

N. microconidia and the *Ilyonectria* species group are the subject of pathogenicity testing in other projects (BS19011 and BS1932). *Clonostachys* species are generally saprophytic on dead or dying plant tissues (i.e. this fungus is unlikely to be a pathogen that is actively causing disease), therefore it would not be recommended for pathogenicity testing.

Sequencing results of members of the *F. solani* complex isolated in this work have shown that there is an array of types/strains of *F. solani* (data not shown) found in the wood of kiwifruit vines, particularly those that are visibly diseased. *F. solani* is a known plant pathogen and it is suggested that a variety of these strains be included in pathogenicity testing.

Although the other five *Fusarium* spp. identified were at low numbers, *F. cerealis* was found regularly and may be a candidate for testing. *Fusarium venenatum* was found at even lower rates but was the major species found in one diseased vine and could also be useful to test.

Recommended groups for pathogenicity testing in Objective 2:

- Fusarium solani complex
- Fusarium cerealis
- Fusarium venenatum

Recommendations for future work

This work aimed to describe the Nectriaceae associated with vine decline of kiwifruit, particularly the incidence and prevalence of each species found. Although 15 species/groups were identified, it is likely that more would be identified if the sample size was larger, and different members of this group will likely be more important in different orchards.

With a sample size of only three orchards, we are unable to draw any conclusions on the principal factors involved in the patterns seen. There are likely to be roles played by scion and rootstock cultivar, rootstock age, region, environment, and previous history of each orchard block.

There is currently no information on the inoculum source or methods of dispersal of any the pathogens identified. The age at which vines are infected, where the infection points are and how each species moves within the vines is unknown. It is likely that each species will be different for each of these factors.

Within the vine decline/decay space, we would suggest initially sampling a larger number of orchards, not only for Nectriaceae, but also for *Neobulgaria alba*, which is likely to have been a part of the fungal community in Orchard 1, and has been previously identified in association with kiwifruit trunk disease.

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Appendix 1 — Orchard maps

Bay	Vine	Row 1	Row 2	Row 3	Row 4	Row 5	Row 6	Row 7	Row 8	Row 9	Row 10	Row 11	Row 12	Row 13	Row 14	Row 15
26	2	D	H	X	D	H	Y	X	H	H	H	H	H	H	H	D
26	1	X	X	H	Y	H	D	D	X	X	H	X	X	X	H	X
25	2	H	D	Y	Y	X	H	X	D	X	H	D	Y	H	D	D
25	1	X	H	X	X	Y	X	H	X	H	X	X	Y	D	X	Y
24	2	H	D	X	D	X	H	X	H	H	D	X	D	Y	H	H
24	1	X	Y	H	X	Y	X	X	X	H 3073.6	Y	Y	X	X	Y	X
23	2	D	H	X	H	H	X	H	H	D	D 3073.16	Y	D	D	D	H
23	1	X	X	D	X	Y	D	H	X	D	X	H	X	H	X	Y
22	2	H	H	X	D	D	H	H	H	X	D	X	D	X	H	X
22	1	X	X	X	H	X	X	X	X	D	Y	Y	Y	H	Y	Y
21	2	H	H	H	X	D	H	D	H	X	X	H	X	D	D	D
21	1	X	X	D	Y	Y	Y	H	X	D	Y	H	X	H	Y	D
20	2	D	D	X	X	H	H	Y	H	H	D	D 3073.15	X	X	H	D
20	1	X	Y	D	Y	X	Y	X	X	H	H 3073.5	D	H	X	X	Y
19	2	H	D	X	Y	H	X	D	H	X	X	Y	X	X	H	D
19	1	X	X	X	X	X	D	H	X	H	X	X	H	D	Y	D
18	2	D	X	H	H	Y	D	X	X	D	H	H 3073.4	D 3073.14	X	H	D
18	1	H	H	Y	Y	X	Y	D	H	X	X	H	H	H	Y	H
17	2	D	H	X	H	H 3073.9	X	H	H	H	Y	X	X	X	H	H
17	1	H	X	D	X	Y	Y	H	X	Y	X	Y	H	H	H	X
16	2	X	H	X	X	D 3073.19	H	X	H	H	H	D	X	H	X	Y
16	1	X	Y	X	H	X	X	X	X	X	Y	X	X	Y	X	X
15	2	H	H	H	X	H	H	H	H	H	H	H	H	H 3073.3	D	H
15	1	X	X	X	H	X	X	Y	X	X	X	Y	X	Y	X	X
14	2	H	H	H	Y	H	H	H	H	H	H	H	H	D 3073.13	H	H
14	1	X	X	X	H	X	X	X	X	Y	X	X	X	Y	X	X
13	2	D	H	D	X	D	H	Y	H	D	H	D	H	H	X	H
13	1	X	X	X	H	X	X	X	X	Y	H	Y	X	X	H	X
12	2	H 3073.10	H	H	X	H	H	H	H	D	X	H	H	H	H	H
12	1	Y	X	X	X	H	Y	Y	X	X	H	X	Y	Y	Y	Y
11	2	D 3073.20	H	H	D	X	X	H	H	D	X	H	X	H	H	D
11	1	H	H	X	X	Y	H	Y	X	X	X	H	H	H	H	Y
10	2	X	X	H	H	H	D	H	H	H	D	D	D	X	X	D
10	1	Y	H	X	H	X	H	Y	X	H	X	Y	Y	H	H 3073.2	Y
9	2	D	X	H	D	H	X	H	H	H	H	D	X	X	X	H
9	1	X	D	H	H	H	X	Y	X	H	H	Y	Y	Y	X	X
8	2	H	X	D	X	D	D	X	H	X	X	D	H	X	D 3073.12	H
8	1	D	H	H	X	D	X	H	X	H	Y	D	H	X	Y	X
7	2	D	D	X	H	Y	H	D	H	X	H	X	X	D	D	H
7	1	X	Y	H	H	X	Y	Y	X	H	X	H	H	X	H	X
6	2	H	D	X	D	D	D 3073.18	D	H 3073.7	D 3073.17	D	D	X	H	X	H
6	1	X	H	X	D	Y	H	Y	X	X	Y	X	H	Y	H	H
5	2	H	D	H	X	H 3073.8	X	X	H	D	H	H	X	D	X	D
5	1	H	H	X	X	X	H	H	X	H	X	D	Y	Y	X	Y
4	2	X	X	H	H	D	X	X	H	X	H	X	D	X	H	H 3073.1
4	1	X	Y	X	Y	Y	X	D	X	Y	Y	X	X	Y	X	H
3	2	H	H	H	H	D	H	X	H	H	H	H	H	H	H	D 3073.11
3	1	X	Y	X	H	Y	H	H	X	X	X	X	X	X	X	Y
2	2	H	X	H	X	X	X	X	H	X	H	H	D	H	H	H
2	1	Y	H	Y	X	X	H	H	H	H	X	X	Y	H	Y	Y
1	2	H	D	H	X	H	X	D	X	H	H	H	H	X	D	D
1	1	Y	X	X	X	Y	H	Y	H	Y	X	H	X	Y	X	Y
Bay	Vine	Row 1	Row 2	Row 3	Row 4	Row 5	Row 6	Row 7	Row 8	Row 9	Row 10	Row 11	Row 12	Row 13	Row 14	Row 15

Figure A1-1. Map of Orchard 1, Paengaroa. Y = young vine, H = asymptomatic, D = visibly diseased, x = missing/removed. Black boxes indicate the sampled vines.

Bay	Row 12	Row 13	Row 14	Row 15	Row 16	Row 17	Row 18	Row 19	Row 20	Row 21	Row 22	Row 23	Row 24
1	D	x	H	x	H	x	H	H	H	D	x	Y	x
2	D	x	H	x	H	x	x	H 3100.5	x	D	H	H	H
3	D	H	H	D	H	H	H	D 3100.15	H	D	x	H	x
4	H	x	H	x	D	x	x	D	x	H	H	H	H
5	D	H	Y	H	H	H	H	H	H	H	x	H	x
6	H	x	H	x	D	x	x	H	x	D	H	H	H
7	H	H	D	D	D	H	H	H	H	H	x	H	x
8	H	x	D	x	H	x	x	D	x	H	H	D 3100.14	D
9	H	H	D 3100.20	H	H	H	H	D	H	H	x	H 3100.4	x
10	H	x	H 3100.10	x	H	x	x	D	x	H 3100.3	H	D	D
11	D	H	D	H	H	H	H	D	H	D	x	H	x
12	D	x	D	x	H	x	x	H	x	D	D	H	H
13	H	H	H	D	H	H	H	H	H	D 3100.13	D (male)	D	x
14	D	x	H	x	H	x	x	H	x	D	H	H	D
15	H	H	H	H	H 3100.6	H	H	H	D	D	x	H	x
16	D	x	H	x	D 3100.16	x	x	H	x	H	H	H	H
17	H	H	H	H	D	H	H	D	H	H	x	D	x
18	H	x	D	x	D	x	x	Y	x	H	H	H	H
19	D	H	H	H	H	H	H	Y	H	D	D (male)	H	x
20	H	x	H	x	H	x	x	H	x	D	H	H	H
21	H	H	H	D	H	H	H	D	H	H	x	H	x
22	H	x	H	x	H	x	x	H	x	H	H	H	H
23	H	H	H	H	H	H	H	H	H	D	x	H	x
24	H	x	D	x	H	x	x	H	x	H	H	H	H
25	Y	H	H 3100.9	D	H	H	H	H	H	H	x	H	x
26	D	x	D 3100.19	x	H	x	x	H	x	H	H	Y	H
27	H	H	H	H	H	H	H	D	H	D	x	H	x
28	H	x	H	x	H	x	x	H	x	H	H	D	H
29	H	H	H	H	H	H	H	H	H	H 3100.2	x	D	x
30	H	x	H	x	H	x	x	D	x	D 3100.12	H	H	H
31	H 3100.8	H	H	H	H	H	H	H	H	H	x	H	x
32	D 3100.18	x	H	x	H	x	x	Y	x	H	H	H	H
33	H	H	Y	H	H	H	H	H	H	D	x	H	x
34	H	x	H	x	H	x	x	Y	x	Y	H	H	H
35	H	H	H	H	H	H	H	H	H	D	x	H	x
36	H	x	H	x	Y	x	x	H	x	H	H	H	H
37	H	H	H	H	H	H	H	H	H	H	x	H 3100.1	x
38	H	x	H	x	H	x	x	H	x	Y	H	D 3100.11	H
39	D	H	H	H	H	D	H	H	H	H	x	H	x
40	H	x	H	x	H	x	x	D 3100.17	x	H	H	H	H
41	H	H	H	H	H	H	H	H 3100.7	H	H	H	H	x
42	H	x	H	x	H	x	x	H	x	x	x	x	x
43	H	H	H	H	H	H	H	H	H	x	x	x	x
44	H	x	H	x	D	H	H	x	x	x	x	x	x
45	H	H	H	H	H	x	x	x	x	x	x	x	x
46	H	x	H	H	x	x	x	x	x	x	x	x	x
47	H	H	H	x	x	x	x	x	x	x	x	x	x
	Row 12	Row 13	Row 14	Row 15	Row 16	Row 17	Row 18	Row 19	Row 20	Row 21	Row 22	Row 23	Row 24

Figure A1-2. Map of Orchard 2, Te Puke. Y = young vine, H = asymptomatic, D = visibly diseased, x = missing/removed. Black boxes indicate the sampled vines.

Vine	Row 68	Row 67	Row 66	Row 65	Row 64	Row 63	Row 62	Row 61	Row 60	Row 59	Row 58	Row 57	Row 56	Row 55	Row 54	Row 53	Row 52	Row 51	Row 50	Row 49	Row 48	Row 47	Row 46
21	H	H	H	H	H	H	H	H 3101.7	H	D	H	D	H	D	H	H	Y	H	H	D	D	H	H
20	H	H	H	H	D	Dead	H	D	D	H	D	H	H	H	H	H	D	H	H	H	D	D	H
19	H	H	H	H	H	H	D	D 3101.17	H	D	H	D	D	H	H	H	H	H	H	H	H	H 3101.1	H
18	H	H	H	D 3101.18	H	H	H	D	H	H	D	D	H	D	H	Y	H	D	Y	H	H	D	D
17	H	Y	H	H 3101.8	D	H	D	H	D	H	D	D	H	H3101.4	D	D	D	H	D	H	H	D 3101.11	H
16	D	H	H	D	D	D	D	H	H	D	H	D	D	D 3101.14	H	D	Y	D	H	D	H	D	H
15	H	H	H	H	D	D	H	H	D	H	H	H	H	D	H	D	D 3101.12	H 3101.2	H	H	H	D	H
14	D	H	H	H	D	D	H	H	H	H	D	H	H	D	H	D	H	D	D	H	H	H	H
13	D	H	H	H	D	H	H	D	H	D	H	H	D	H	D	D	D	D	D	H	D	H	H
12	D	D	H	H	D	H	D	H	D	H	H	H	D	H	D	D	D	H	H	D	H	Y	H
11	D	D	H	H	H	H	H	H	D 3101.16	H 3101.6	H	D	H	D	D	D	D	D	H	H	D	D	D
10	H	D	H	H	H	H	H	D	D	D	H	D	D	H	H	H 3101.3	D	D	D	D	D	H	H
9	H	D	H	H	H	H	H	H	H	H	H	H	D	H	D	D	D 3101.13	D	D	H	H	H	H
8	D	H	H	H	D	H	H	D	Y	D	H	H	D	H	D	D	D	D	H	H	H	H	H
7	D	Y	H	H	D	H	D 3101.20	H	H	D	H	H	H	H	H	H	D	H	H	H	H	D	H
6	D	D 3101.19	H	H	H	H	H 3101.10	H	H	D	H 3101.5	H	H	H	H	D	H	D	H	H	D	H	H
5	H	H 3101.9	D	H	H	D	H	H	H	H	D 3101.15	D	D	H	H	D	D	H	D	H	D	H	H
4	H	D	D	H	H	H	H	H	H	H	H	D	D	H	D	D	D	H	H	H	H	H	H
3	D	H	H	H	D	H	D	Y	H	H	H	D	H	H	H	H	D	H	H	D	H	H	H
2	H	H	H	H	H	H	H	H	H	H	D	H	H	H	H	H	H	D	D	H	D	H	H
1	H	D	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	D	H	H	H	H	H
Vine	Row 68	Row 67	Row 66	Row 65	Row 64	Row 63	Row 62	Row 61	Row 60	Row 59	Row 58	Row 57	Row 56	Row 55	Row 54	Row 53	Row 52	Row 51	Row 50	Row 49	Row 48	Row 47	Row 46

Figure A1-3. Map of Orchard 3, Motueka. Y = young vine, H = asymptomatic, D = visibly diseased, x = missing/removed. Black boxes indicate the sampled vine.

Appendix 2 — Vine maps

The following figures represent each individual isolation at each sampling site on 20 vines from each orchard. These are able to be “zoomed in” on.

The identifications focussed on members of the Nectriaceae, e.g. *Fusarium* species, and members of the *Cylindrocarpon* complex. Members of the Nectriaceae have been coloured in the figures. Isolation points from which no fungi grew are shown as “NG” and are greyed-out. The fungal names of common saprophytes or contaminants, such as Penicillium and Mucor are also greyed-out in the figures.

Isolation points that are shown as “other” represent fungi that are not members of the Nectriaceae, however, *Phomopsis*, *Botryosphaeria* and *Neofabraea* are named as these are the three most common fruit rot fungi (coloured dark grey) and this may be useful information.

The *Fusarium solani* complex is shown as *F. solani*.

		'healthy' vines										'diseased' vines									
	core position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
100 cm woody core	a	other	other	NG	other	NG	NG	Mucor	Penicillium	Phomopsis	Cladosporium	Phomopsis	Phomopsis	other	NG	Botryosphaeria	Phomopsis	other	Ilyonectria sp., Bot	other	Ilyonectria sp., Pho
	b	Cladosporium	NG	NG	other	NG	other	other	NG	Phomopsis	I. europaea	Phomopsis	Phomopsis	I. robusta	NG	Neo. microconidia	Phomopsis	other	Ilyonectria sp.	other	Ilyonectria sp.
	c	Cladosporium	NG	NG	NG	NG	other	other	NG	Neofabraea actinid	Trichoderma	Phomopsis	Phomopsis	I. robusta	NG	Trichoderma	NG	other	Ilyonectria sp.	other	Epicoccum
	d	NG	NG	other	NG	NG	NG	other	NG	Phomopsis	NG	Phomopsis	Phomopsis	I. robusta	NG	other	Phomopsis	other	Ilyonectria sp.	other	other
	e	other	NG	Cladosporium	NG	NG	NG	other	other	NG	Neo. microconidia	NG	NG	NG	Cladosporium	other	other	other	Ilyonectria sp.	Cladosporium	I. europaea
	f	other	NG	NG	NG	NG	NG	other	other	NG	Neo. microconidia	NG	NG	NG	Cladosporium	other	NG	other	other	NG	I. europaea
	g	other	NG	NG	Neofabraea actinid	Penicillium	other	other	NG	other	Neo. microconidia	other	NG	NG	other	other	NG	other	other	NG	other
	h	other	NG	other	NG	NG	other	other	NG	NG	NG	NG	NG	F. solani	NG	other	NG	F. solani	other	Penicillium	Ilyonectria sp.
	i	NG	other	NG	other	NG	F. solani	other	other	other	I. europaea	NG	NG	F. solani	NG	Cladosporium	NG	F. solani	other	Cladosporium	Ilyonectria sp.
	j	NG	NG	NG	other	NG	F. solani	other	NG		I. europaea	NG	NG	NG	NG	NG	NG	F. solani	Clonostachys sp.	other	Ilyonectria sp.
	k	NG	other	NG	other	NG	F. solani	other	other		I. europaea	NG	NG	F. solani	other	NG	NG	F. solani	other	NG	Ilyonectria sp.
	l	NG	NG	other		NG	other	NG	NG			NG	NG	NG	other	NG	NG	F. solani	NG	Ilyonectria sp., F. s	Ilyonectria sp.
	m	NG	Botryosphaeria			Phomopsis	NG	other	NG			NG	other	other	Neo. microconidia	Cladosporium	NG	I. robusta	Clonostachys sp.	Penicillium	Botryosphaeria
	n	NG	other			NG	Phomopsis	other	NG				NG	other	other	Cladosporium	other	I. robusta			
	o		Botryosphaeria			NG		Mucor					NG		Phomopsis	NG	NG	I. robusta			
	p		Botryosphaeria			Phomopsis		Phomopsis					NG			NG	NG				
	q												NG				NG				
	r												NG								
	s												Cladosporium								
	t												NG								
30 cm woody core	a	Trichoderma	Phomopsis	NG	Cladosporium	Phomopsis	Mucor	other	other	other	NG	other	other	Phomopsis	other	Mucor	other	other	Thelonectria sp.	I. robusta	I. europaea
	b	Trichoderma	Phomopsis	NG	Cladosporium	Phomopsis	other	NG	Clonostachys sp.	NG	NG	NG	other	I. robusta	other	NG	NG	NG	Clonostachys sp.	Clonostachys sp.	I. europaea
	c	other	other	Neo. microconidia	NG	NG	other	other	other	NG	NG	other	other	I. robusta	other	other	other	NG	F. solani	other	other
	d	other	other	Neo. microconidia	NG	Phomopsis	Mucor	NG	other	NG	I. europaea	other	other	I. robusta	Trichoderma	Cladosporium	other	Cladosporium	F. solani	other	NG
	e	other	F. solani	Neo. microconidia	Ilyonectria sp.	NG	F. solani	other	NG	other	I. europaea	other	other	F. solani	F. venenatum	NG	other	other	other	Cladosporium	NG
	f	Cladosporium	F. solani	Neo. microconidia	NG	NG	F. solani	other	NG	NG	NG	other	I. europaea	F. solani	F. venenatum	NG	other	other	Trichoderma	NG	other
	g	other	F. solani	Neo. microconidia	NG	other	other	other	I. europaea	NG	NG	Cladosporium	I. europaea	other	F. venenatum	NG	other	Cladosporium	Trichoderma	NG	other
	h	other	Cladosporium	NG	NG	NG	F. cerealis	other	I. europaea	NG	NG	other	other	other	F. venenatum	NG	NG	other	Trichoderma	Penicillium	NG
	i	NG	Phomopsis		NG	NG	F. cerealis	NG	Mucor	NG	other	NG	other	other	F. venenatum	NG	NG	other	Trichoderma	other	NG
	j	NG	other		Mucor	other	F. cerealis	NG	NG			NG	other	F. solani	F. venenatum	NG	NG	NG	Trichoderma	other	NG
	k	other	Phomopsis		Mucor	NG		NG	Mucor			other	other	F. solani	Trichoderma	Penicillium	other	NG	Trichoderma	Neo. microconidia	NG
	l	other	Phomopsis		Botryosphaeria, PH	NG		NG	Mucor			Cladosporium	other	F. solani	Trichoderma	NG	NG	Cladosporium	Trichoderma	other	I. torresensis, Botry
	m	other				NG		NG				Cladosporium	other	F. solani	other	NG	NG	other	other	other	I. torresensis
	n	other						NG				Cladosporium	other	other	other	Cladosporium	other	other	I. robusta	other	I. torresensis
	o											NG	other	Ilyonectria sp.	other	Trichoderma	NG	other		NG	Botryosphaeria
	p											NG	NG		NG	other	other			NG	
	q												NG		other	Cladosporium				NG	
	r												NG		other	NG					
	s												other			NG					
	t															Cladosporium					
	u															NG					
	v															NG					
bark samples from crown	a	NG	other	other	Phomopsis	other	other	NG	Trichoderma	Epicoccum	Mucor	Clonostachys sp.	Clonostachys sp.	other	Neo. microconidia	Ilyonectria sp.	Mucor	NG	Mucor	I. robusta	Mucor
	b	NG	I. robusta	NG	Phomopsis	NG	other	other	Trichoderma	other	other	F. solani	Clonostachys sp.	NG	NG	Ilyonectria sp.	other	other	Mucor	I. robusta	I. robusta
	c	NG	I. robusta	NG	Phomopsis	NG	NG	Clonostachys sp.	Trichoderma	other	Mucor	Ilyonectria sp.	F. solani	Phomopsis	NG	Ilyonectria sp.	other	other	other	I. robusta	I. robusta
	d	other	I. robusta	NG	Phomopsis	NG	NG	Clonostachys sp.	Trichoderma	other	other	Clonostachys sp.	Mucor	NG	Phomopsis	Ilyonectria sp.	Ilyonectria sp.	other	other	I. robusta	I. robusta
	e	NG			other			Penicillium			other		Clonostachys sp.					Mucor	F. cerealis	I. robusta	Mucor
	f	other			other			other			Ilyonectria sp.		F. solani, Clonostachys sp.					Mucor	other	I. robusta	F. equiseti, Ilyonec
	g	other			F. venenatum			NG			Ilyonectria sp.		Clonostachys sp.					NG	Mucor	I. robusta	F. equiseti, Ilyonec
	h	other			other			Mucor					Clonostachys sp.					I. robusta	other	I. robusta	Trichoderma
	i				other			Penicillium											other	I. robusta	Ilyonectria sp.
	j				F. venenatum			other											other	other	Ilyonectria sp.
	k							other											other	other	Trichoderma
	l							Mucor											other		
	m							Mucor													

Figure A2-1. Orchard 1 (Paengaroa). Results of isolations from each vine at each sampling site - bark from the crown, and wood cores at 30 cm and 100 cm above the soil. Isolations were made at 1 cm intervals across the wood cores. Vines 1–10 were largely asymptomatic, vines 11–20 were visibly diseased.

100 cm woody core

core position	'healthy' vines										'diseased' vines									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
a	Phomopsis	Clonostachys sp.	other	Phomopsis	Botryosphaeria	other	other	Phomopsis	Phomopsis	Phomopsis	other	Phomopsis	Phomopsis	other	F. cerealis	I. robusta	Phomopsis	Phomopsis	other	Phomopsis
b	Phomopsis	NG	other	other	Botryosphaeria	other	other	Phomopsis	Phomopsis	Phomopsis	other	Phomopsis	other	other	F. cerealis	I. robusta	Phomopsis	Phomopsis	other	Phomopsis
c	other	NG	NG	NG	other	Penicillium	NG	Phomopsis	Phomopsis	Phomopsis	NG	Phomopsis	other	Ilyonectria sp.	Ilyonectria sp.	NG	F. solani	NG	NG	NG
d	other	other	Penicillium	NG	other	other	other	NG	Phomopsis	NG	NG	Phomopsis	NG	other	Ilyonectria sp.	NG	F. oxysporum	other	NG	NG
e	other	Mariannea sp., Fus	NG	NG	other	other	NG	NG	Phomopsis	other	other	NG	NG	other	Ilyonectria sp.	Phomopsis	other	NG	Phomopsis	other
f	other						Phomopsis	Phomopsis	Phomopsis	NG		NG		other	Ilyonectria sp.		other	other	Phomopsis	other
g								Phomopsis		Phomopsis		Phomopsis		Phomopsis, other	other				Phomopsis	
h												Phomopsis								
i																				
j																				
k																				
l																				
m																				
n																				
o																				
p																				
q																				
r																				
s																				
t																				

30 cm woody core

a	Phomopsis	Cladosporium	Clonostachys sp.	other	Clonostachys sp.	F. solani	Phomopsis	Phomopsis, other	Mucor	F. oxysporum	other	Neo. microconidia	NG	Clonostachys sp.	other	Clonostachys sp.	F. solani	F. solani	other	NG
b	NG	other	Clonostachys sp.	other	F. equiseti	F. solani	other	Phomopsis	Mucor	other	Clonostachys sp.	Neo. microconidia	NG	Clonostachys sp.	other	Phomopsis, Clonos	F. solani	F. solani	other	NG
c	Clonostachys sp.	Penicillium	Neo. microconidia	other	Fusarium sp.	F. solani	other	other	I. europaea	other	F. solani	Neo. microconidia	other	Clonostachys sp.	other	other	F. solani	F. solani	other	other
d	Clonostachys sp.	other	Neo. microconidia	other	NG	F. solani	other	I. europaea	I. europaea	other	F. solani	Neo. microconidia	other	Clonostachys sp.	other	other	F. solani	F. solani	other	other
e	Neo. microconidia	other	Neo. microconidia	NG	other	F. solani	other	other	I. europaea	other	F. solani	Neo. microconidia	F. solani	Clonostachys sp.	other	F. solani	F. solani	F. solani	other, Clonostachy	NG
f	Neo. microconidia	other	Neo. microconidia	NG	other	F. solani	other	other	other	other	F. solani	Neo. microconidia	F. solani	Clonostachys sp.	other	F. solani	F. solani	F. solani	other, Clonostachy	NG
g	Neo. microconidia	other	Neo. microconidia	Neo. microconidia	Neo. microconidia	F. solani	F. venenatum	NG	F. solani	Clonostachys sp.	F. solani	NG	Ilyonectria sp.	F. solani	other	F. solani	F. solani	F. solani	Clonostachys sp.	F. solani
h	Neo. microconidia	other	Neo. microconidia	other	Clonostachys sp.	F. solani	F. solani	NG	F. solani	Clonostachys sp.	F. solani	NG	other	F. solani	other	F. solani	F. solani	F. solani	Clonostachys sp.	F. solani
i	Neo. microconidia	other	Neo. microconidia	other	I. europaea	F. solani	Neo. microconidia	other	F. solani	Clonostachys sp.	F. solani	Ilyonectria sp.	Neo. microconidia	F. solani	Ilyonectria sp., Clo	F. solani	F. solani	F. solani	Clonostachys sp.	F. solani
j	Neo. microconidia	other	other	other	I. europaea	F. solani	Neo. microconidia	NG	F. solani	Clonostachys sp.	other	Ilyonectria sp.	Neo. microconidia	F. solani	Ilyonectria sp., Clo	F. solani	F. solani	F. solani	Clonostachys sp.	F. solani
k	other	other	other	other	I. europaea	F. solani	other	NG	other	Penicillium		Ilyonectria sp.	Neo. microconidia	F. solani	Clonostachys sp.	F. solani	F. solani	NG	Neo. microconidia	F. solani
l	Clonostachys sp.	other	other	Clonostachys sp.	I. europaea	F. solani	other	NG	other	other			Neo. microconidia	F. solani		F. solani	F. solani	NG	Clonostachys sp.	F. solani
m	Phomopsis	other			Clonostachys sp.	Clonostachys sp.	other	other	other	F. oxysporum			Neo. microconidia	Phomopsis, Clonostachys sp.		F. solani	F. solani	NG	Clonostachys sp.	F. solani
n		other			Clonostachys sp.		other	other		F. oxysporum				Phomopsis, Clonostachys sp.		F. solani	other	NG	Clonostachys sp.	F. solani
o		other						other		Penicillium				Phomopsis, Clonostachys sp.		F. solani	other	NG	Clonostachys sp.	F. solani
p		other						other								F. solani	other, Clonostachy	Clonostachys sp.	Clonostachys sp.	F. solani
q								other								other	other, Clonostachys sp.		NG	F. solani
r								other								Clonostachys sp.			NG	F. solani
s																				NG
t																				NG
u																				NG
v																				NG

bark samples from crown

a	Clonostachys sp.	other	Neo. microconidia	Penicillium	other	other	other	Clonostachys sp.	Mucor	Clonostachys sp.	other	other	NG	Clonostachys sp.	Clonostachys sp.	Clonostachys sp.	F. solani	other	Clonostachys sp.	other
b	Clonostachys sp.	F. oxysporum	Neo. microconidia	NG	other	other	other	Clonostachys sp.	Mucor	Clonostachys sp.	F. venenatum, oth	F. solani	F. solani	other	other	F. solani	other	F. solani	other, Clonostachy	Clonostachys sp.
c	Clonostachys sp.	F. oxysporum	Mucor	Fusarium sp.	other	other	Clonostachys sp.	Clonostachys sp.	Mucor	Mucor	other	Fusarium sp.	NG	Fusarium sp., Clon	other	F. solani	Ilyonectria sp., F. s	F. solani	other	F. solani
d	Phomopsis	Ilyonectria sp.	Mucor	other	other	other	Clonostachys sp.	Clonostachys sp.	Mucor	Mucor	F. venenatum	Fusarium sp.	Clonostachys sp.	NG	other	F. solani	other	Fusarium sp.	Clonostachys sp.	other
e											other									
f																				
g																				
h																				
i																				
j																				
k																				
l																				
m																				

Figure A2-2. Orchard 1 (Te Puke). Results of isolations from each vine at each sampling site - bark from the crown, and wood cores at 30 cm and 100 cm above the soil. Isolations were made at 1 cm intervals across the wood cores. Vines 1–10 were largely asymptomatic, vines 11–20 were visibly diseased.

		'healthy' vines										'diseased' vines									
	core position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
canker	a	-	-	-	-	-	-	-	-	-	-	other	Phomopsis	Phomopsis	I. liriodendri	Neo. microconidia	Neo. microconidia	Phomopsis	Neo. microconidia	Clonostachys sp.	Clonostachys sp.
	b	-	-	-	-	-	-	-	-	-	-	Fusarium sp.	Phomopsis	Neo. microconidia	Neo. microconidia	Neo. microconidia	Neo. microconidia	Neo. microconidia	Neo. microconidia	Fusarium sp.	Clonostachys sp.
	c	-	-	-	-	-	-	-	-	-	-	other	Neo. microconidia	Neo. microconidia	Neo. microconidia	Neo. microconidia	Neo. microconidia	Neo. microconidia	NG	Neo. microconidia	NG
	d	-	-	-	-	-	-	-	-	-	-	other	Neo. microconidia	Neo. microconidia	F. solani	Neo. microconidia	F. solani, I. robusta	Neo. microconidia	Neo. microconidia	Neo. microconidia	Neo. microconidia
	e	-	-	-	-	-	-	-	-	-	-	other	Neo. microconidia	Neo. microconidia	Neo. microconidia	Neo. microconidia	Neo. microconidia	Neo. microconidia	Mucor	Neo. microconidia	Neo. microconidia
	f	-	-	-	-	-	-	-	-	-	-	other	Neo. microconidia	Phomopsis	Neo. microconidia	Neo. microconidia	Neo. microconidia	Neo. microconidia	Neo. microconidia	Neo. microconidia	Neo. microconidia
	g	-	-	-	-	-	-	-	-	-	-	Botryosphaeria, ot	Neo. microconidia			Neo. microconidia	Phomopsis	Neo. microconidia	Neo. microconidia	other	Neo. microconidia
	h	-	-	-	-	-	-	-	-	-	-		Phomopsis			Neo. microconidia	Neo. microconidia	Phomopsis	Neo. microconidia	Neo. microconidia	Neo. microconidia
	i	-	-	-	-	-	-	-	-	-	-								Neo. microconidia	Clonostachys sp.	Neo. microconidia
	j	-	-	-	-	-	-	-	-	-	-								Neo. microconidia		Neo. microconidia
	k	-	-	-	-	-	-	-	-	-	-								Mucor		Phomopsis
100 cm woody core	a	Phomopsis	F. cerealis	other	Clonostachys sp.	Neo. microconidia	Penicillium	other	Clonostachys sp.	Clonostachys sp.	other, Clonostachy	other	F. solani	F. equiseti	Clonostachys sp., I	other	other	other	other	other	Neo. microconidia
	b	other	Clonostachys sp.	other	Clonostachys sp.	Neo. microconidia	Penicillium	other	Clonostachys sp.	other	other	other	other, Clonostachy	F. equiseti	Clonostachys sp., I	Phomopsis, other	other	F. equiseti	other	Neo. microconidia	Neo. microconidia
	c	other	other	other	Clonostachys sp.	Neo. microconidia	Penicillium	NG	I. robusta	other	other	other	other	other	Clonostachys sp.	other	Clonostachys sp.	Clonostachys sp.	other	NG	Clonostachys sp.
	d	other	other	other	Clonostachys sp.	other, Aspergillus	Clonostachys sp.	Neo. microconidia	I. robusta	Clonostachys sp.	other	Phomopsis	F. solani	other	Neo. microconidia	other	other	other	Fusarium sp.	Epicoccum	Neo. microconidia
	e	I. europaea	other	other	NG	Ilyonectria sp.	Trichoderma	Neo. microconidia	Phomopsis		Clonostachys sp.	-	Phomopsis, Clono	other	Neo. microconidia	other	other	other	Ilyonectria sp.	other	Neo. microconidia
	f	Phomopsis	Clonostachys sp.	other	other	Ilyonectria sp.	I. liriodendri				other		other	other	Neo. microconidia	other, Clonostachys sp.	other	I. europaea	Ilyonectria sp.	other	Clonostachys sp.
	g		F. solani		other		I. liriodendri								Neo. microconidia			I. europaea	Neo. microconidia		
	h		Clonostachys sp.				I. liriodendri								NG			Phomopsis	I. liriodendri		
	i		F. solani, Clonostachys sp.				F. avenaceum								NG				I. liriodendri		
	j		Clonostachys sp.				F. avenaceum								Clonostachys sp.				other		
	k		Clonostachys sp.				F. avenaceum												I. liriodendri		
	l		Clonostachys sp.																I. liriodendri		
	m		Clonostachys sp.																I. liriodendri		
	n		Clonostachys sp.																I. liriodendri		
	o																		Phomopsis		
	p																		Phomopsis		
	q																				
	r																				
	s																				
	t																				
30 cm woody core	a	Epicoccum	Epicoccum	I. liriodendri	other	Penicillium	other, Clonostachy	Penicillium	other	other	other	Neo. microconidia	Clonostachys sp.	F. avenaceum, I. li	Botryosphaeria, Cl	other	other	other	other	other	F. cerealis
	b	other	other	I. liriodendri	other	Alternaria	other	Mucor	other	other	Penicillium	Neo. microconidia	Clonostachys sp.	I. liriodendri	Botryosphaeria, Cl	other	other	other	other	other	Neo. microconidia
	c	other	other	NG	Cladosporium	Clonostachys sp.	Epicoccum	other	other	other	Clonostachys sp.	NG	Clonostachys sp.	I. liriodendri	other, Clonostachy	other	other	Clonostachys sp.	NG	Clonostachys sp.	Neo. microconidia
	d	Phomopsis	other	I. liriodendri	other	Clonostachys sp.	other	other	other	other	Clonostachys sp.	F. solani	Clonostachys sp.	I. liriodendri	Phomopsis	other	Mucor	Penicillium	NG	Clonostachys sp., E	Neo. microconidia
	e	NG	NG	other	other	other	other	Mucor	other	Thelonectria sp.	Clonostachys sp.	F. solani	Clonostachys sp.	I. liriodendri	Clonostachys sp.	Clonostachys sp.	other	Ilyonectria sp.	other	Clonostachys sp., E	other, Clonostachy
	f	I. liriodendri	Phomopsis	other	other	other	Clonostachys sp.	Mucor	other	other	Clonostachys sp.	F. solani	Clonostachys sp.	I. liriodendri	Clonostachys sp.	Clonostachys sp.	other	Ilyonectria sp., oth	other	Epicoccum	other, Clonostachy
	g		F. solani	other	other	Clonostachys sp.	Clonostachys sp.	other	other	other	Clonostachys sp.	Fusarium sp.	other, Clonostachy	I. liriodendri	Clonostachys sp.	other	other	F. oxysporum	other	other	other, Clonostachy
	h		F. solani	other	other	other	other	other	other	other	Clonostachys sp.	F. cerealis	other, Clonostachy	I. liriodendri	other	Clonostachys sp.	F. cerealis	other, Clonostachy	other	other	other, Clonostachy
	i		Penicillium	other	NG	other	other, Clonostachy	Ilyonectria sp., oth	other	other	Clonostachys sp.	Clonostachys sp.	other, Clonostachy	I. liriodendri, other	Clonostachys sp.	F. cerealis	other	other	NG	other	Clonostachys sp.
	j		Neo. microconidia	other	Neo. microconidia	other	F. cerealis	Mucor	other	other	Clonostachys sp.	F. solani	other, Clonostachy	I. liriodendri	Clonostachys sp.	Fusarium sp.	Penicillium	Fusarium sp., othe	other	other	other
	k		Neo. microconidia	other	NG	other	other	Fusarium sp.	other	other	other	F. solani	Clonostachys sp.	I. liriodendri	Clonostachys sp.	NG	other, Penicillium	other	Penicillium	other	F. solani
	l		Penicillium	other	NG	Epicoccum	other		other	F. solani	Pithomyces	F. solani	other, Clonostachy	I. liriodendri	other	NG	other	Ilyonectria sp.	NG	other	F. solani
	m		other	other	Clonostachys sp.	I. liriodendri	Clonostachys sp.		Fusarium sp.	F. oxysporum	Fusarium sp.	NG			other	Ilyonectria sp.	Clonostachys sp.	F. cerealis	other	Clonostachys sp.	Phomopsis
	n		Clonostachys sp.	other		Clonostachys sp.	other			NG	Clonostachys sp.	other			Clonostachys sp.	Ilyonectria sp.	Clonostachys sp.	other	Neo. microconidia	I. europaea	F. cerealis
	o		Clonostachys sp.	other		NG	Clonostachys sp.			Clonostachys sp.		Mucor			other	other		other	Mucor	Clonostachys sp.	F. cerealis
	p			other		Clonostachys sp.				Clonostachys sp.					Clonostachys sp.	NG		other	other	Clonostachys sp.	F. cerealis
	q			F. avenaceum		NG									Penicillium	other			other	Ilyonectria sp.	Clonostachys sp.
	r			F. avenaceum		NG													other		Clonostachys sp.
	s			Ilyonectria sp.		I. liriodendri													F. cerealis		
	t					NG													F. cerealis		
	u																		Ilyonectria sp., Clonostachys sp.		
	v																				
bark samples from crown	a	other	F. cerealis	other	other	other	other	other	Clonostachys sp.	Clonostachys sp.	other	Clonostachys sp.	other	Ilyonectria sp., Clo	other	F. cerealis	I. robusta	other	Penicillium	Fusarium sp.	F. venenatum
	b	Phomopsis	F. cerealis	Penicillium	other	Phomopsis, Clono	other	other	Clonostachys sp.	Ilyonectria sp., Clo	other	Clonostachys sp.	other	Ilyonectria sp., Clo	other	other	I. robusta	other	Penicillium	F. avenaceum	F. venenatum
	c	other	NG	other	Neo. microconidia	Clonostachys sp.	Penicillium	Fusarium sp.	Phomopsis, other	F. solani	other	other	other	Ilyonectria sp., Clo	other	other	I. robusta	I. robusta	Dactylonectria sp.	Clonostachys sp.	NG
	d	other	NG	NG	Neo. microconidia	Phomopsis	Penicillium	other	other	other	other	other	other	Ilyonectria sp., Clo	other	Mucor	I. robusta, Clonost	other	other	Clonostachys sp.	NG
	e	Phomopsis		other		other			other	Clonostachys sp.	other	Neo. microconidia	other	Clonostachys sp.				other		Neo. microconidia	
	f	other		Neo. microconidia					I. robusta	Clonostachys sp.	other	Clonostachys sp.	other	Clonostachys sp.				other		Neo. microconidia	
	g	other							other											I. europaea	
	h	other							Neo. microconidia											I. europaea	
	i																				
	j																				
	k																				
	l																				
	m																				

Figure A2-3. Orchard 3 (Motueka). Results of isolations from each vine at each sampling site - bark from the crown, wood cores at 30 cm and 100 cm above the soil and from cankers on the leader (if present). Isolations were made at 1 cm intervals across the wood cores. Vines 1–10 were largely asymptomatic, vines 11–20 were visibly diseased.

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