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Identification and characterization of *Ilyonectria* species associated with root rot of kiwifruit

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Executive summary

Identification and characterization of *Ilyonectria* species associated with root rot of kiwifruit

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Species of *Ilyonectria* and *Dactlyonectria* genera cause root rots of a wide range of plants including kiwifruit. In other countries infection of kiwifruit by these pathogens causes leaf wilting, black and necrotic lesions on woody tissues of both the rootstock and roots, and death of the trees. The aims of this research were to (1) use DNA sequencing to identify the *Ilyonectria* species that have been isolated from kiwifruit in New Zealand, (2) determine how pathogenic they are on detached kiwifruit root material and (3) determine if isolates from grapevine are pathogenic on kiwifruit and therefore a potential disease reservoir. This report details the results relevant to aims 2 and 3, with previous reports detailing the identity of *Ilyonectria* species associated with kiwifruit.

In the current report the pathogenicity of 15 isolates comprising members of the species *Ilyonectria europaea, I. liriodendri, I. robusta* and *Dactylonectria macrodidyma* were assessed for their ability to cause lesions and colonise wounded roots of *Actinidia* sp. This experiment was repeated thrice. All four species produced lesions on roots of *Actinidia* sp. that were significantly greater than those in the control treatment. The roots were discoloured, presenting morphology typical of infection by these species. The size of the lesions did not differ between the pathogen species, nor reflect the host plant (*Actinidia* sp. or *Vitis* sp.) from which they were originally sourced, however, there were small differences between the lesion sizes for some isolates within a particular pathogen species.

All four pathogen species endophytically colonised the root tissue, some in advance of the lesion. In experiments 2 and 3 there was some evidence that species/isolates recovered from *Vitis* sp. were less able to endophytically colonise *Actinidia* sp. roots, however, this was very minor (7% of roots). There was some variation in the colonisation ability between isolates within a species.

Overall data show that all four species are able to infect wounded roots, produce lesions and move endophytically within the root tissue of *Actinidia* sp., and that this infective potential was irrespective of whether the isolates were originally sourced from an *Actinidia* sp. or *Vitis* sp. host.

The pathogenicity data, and previous work on kiwifruit trunk diseases (KTDs), indicate that the species from kiwifruit are part of a complex of fungal pathogens associated with vine decay symptoms in kiwifruit. Further work on these isolates in whole plants would aid in confirming their association with symptomology.

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1 Introduction

Root rots caused by pathogenic fungal species within the *Cylindrocladiella, Ilyonectria* and *Dactylonectria* genera can result in significant damage to a wide range of high-value crops such as grape, avocado and kiwifruit. Studies investigating root and trunk diseases in Actinidia sp. overseas have reported an association between *Ilyonectria* spp. (synonym *Cylindrocarpon* spp.) and symptomatic vines. This was first recorded by Gianetti et al. (2002) who identified *Cylindrocarpon* sp. in association with wood decay of kiwifruit in Piedmonte, Italy, in 1996, when it was found with a group of fungi including *Fusarium solani*.

In 2003, Nipoti et al. also reported an unusual wood disease of kiwifruit in Italy. The vines were characterised by abnormal trunk thickening and marked discolouration of the annual growth rings. Vines with these symptoms also had compromised foliar growth, which progressively worsened. The most frequent fungi isolated from the symptomatic roots and trunk sections were *Acremonium* spp., *Cylindrocarpon* spp., *Fusarium* spp., *Phaeoacremonium* spp., *Phialophora* spp. and *Phomopsis* spp. (Nipoti et al. 2003). When isolations were made from the symptomatic vines during spring, autumn, and winter, *Cylindrocarpon* spp. were also recovered from trunks and branches (particularly in spring time), and were found consistently in the roots during all periods (Nipoti et al. 2003).

Later, Erper et al. (2011) identified *Ilyonectria liriodendri* (synonym *Cylindrocarpon liriodendri*) from kiwifruit vines in the Black Sea region of Turkey. Symptoms included necrotic lesions on woody tissues of both the rootstock and roots. In 2013, Erper et al. further described the kiwifruit root rot disease observed in 2009-2010 in Turkey and identified six fungal species from necrotic roots and crown sections of symptomatic uprooted vines; *Dactylonectria pauciseptata* (synonym *Cylindrocarpon pauciseptatum*)*, Cylindrocladiella parva* (synonym *Cylindrocarpon parva*)*, Ilyonectria europaea, I. liriodendri, I. robusta* and *I. torresensis* (Erper et al. 2013). When very young *Actinidia chinensis* var*. deliciosa* 'Hayward' seedlings were inoculated with *isolates of these six fungal species*, 91% of the isolates were pathogenic (Erper et al. 2013).

The published records of members of the *Ilyonectria* group associated with root and trunk diseases of *Actinidia* spp. worldwide are summarised in Table 1.

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

Species	Country	Reference
Dactylonectria pauciseptata (as *Cylindrocarpon pauciseptatum*)	Turkey	Erper et al. (2013)
Ilyonectria europaea	Turkey	Erper et al. (2013)
Ilyonectria liriodendra	Turkey	Erper et al. (2011), Erper et al. (2013)
Ilyonectria robusta	Turkey	Erper et al. (2013)
Ilyonectria torresensis	Turkey	Erper et al. (2013)
*'Cylindrocarpon** spp.'	Italy	Gianetti et al. (2002), Nipoti et al. (2003)

*Identified by the taxonomic criteria of 2002/2003, these isolates have not been reclassified with the new taxonomic criteria.

There are currently a limited number of reports of *Ilyonectria* spp. (and synonyms) from kiwifruit in New Zealand, none of which are published. The first report of *Ilyonectria* spp. (as synonym *Cylindrocarpon* sp.) being associated with kiwifruit vines in New Zealand came from isolations from four orchards with trunk disease in 2006 (Manning et al. 2007). Later in 2007, 11 orchards with trunk

disease were surveyed and core samples of wood were taken from symptomatic and asymptomatic *A. chinensis* var. *deliciosa* 'Hayward' and *A. chinensis* var. *chinensis* 'Hort16A'. A variety of pathogens were isolated from the woody samples, including *Neobulgaria alba* (synonym *Phialophora alba*), *Verticillium dahliae* and *Phytophthora cryptogea*. *Ilyonectria* spp. were isolated from both cultivars from orchards in Te Puke; one of the isolations (MM431) was identified as *I. liriodendri* by sequencing of the ITS gene region (Manning et al. 2009). Pathogenicity testing was done by on-orchard cane inoculations, using mycelial plugs of five fungal species isolated in the 2007 survey, including MM431 (*I. liriodendri*). All five fungi were able to be recovered from canes after 8 months, with the longest lesions recorded from those inoculated with *N. alba* (c. 21 mm), *Phoma* sp. (c. 22 mm) and *I. liriodendri* (c. 24 mm) (Manning et al. 2009; Manning et al. 2010).

Since the first reported observations of *Ilyonectria* spp. in symptomatic kiwifruit vines were made in New Zealand (2006), these fungi have become more frequently noted in orchard surveying, though all of these observations are unpublished to date. Isolations have been made of *Ilyonectria* spp. from Kerikeri in 2016, Te Puke in 2017, Edgecumbe and Pyes Pa in 2018, and Kerikeri, Paengaroa, Te Puke and Motueka in 2019. During a delineating survey for *Neonectria microconidia* in the upper South Island in early 2019, *Ilyonectria* spp. were found from 7 orchards in Brooklyn, Riwaka, Nelson and Motueka (unpub. data).

The records of members of the *Ilyonectria* group associated with root and trunk diseases of *Actinidia* spp. In New Zealand are summarised in Table 2. Collectively, alongside older reports and evidence, these more recent and numerous reports of *Ilyonectria* spp. in kiwifruit in New Zealand provide justification for accurate and informative pathogenicity testing of these fungi in order to determine the potential level of threat they pose to the kiwifruit industry.

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

Fungal species	Region	Reference
Ilyonectria spp.	Bay of Plenty	Manning et al. (2007), Manning et al. (2009)
Dactylonectria sp.	Bay of Plenty, Motueka	MPI (2018a, b), Tyson et al. (2020a)
Ilyonectria liriodendra	Bay of Plenty	(Manning et al. 2009)
Ilyonectria robusta	Bay of Plenty	(MPI 2018b), Tyson et al. unpub. 2017
Ilyonectria sp.	Bay of Plenty, Kerikeri, Motueka	(MPI 2017, 2018b), Tyson et al. (2020b), Tyson et al. unpub.

This project (BS1932 *Identification and characterization of* Ilyonectria *species associated with root rot of kiwifruit*) aimed to clarify the species of *Ilyonectria* associated with kiwifruit in New Zealand and establish whether they were pathogenic. In objective 1 (*To correctly identify the isolates already recovered from kiwifruit using the new taxonomic process*) the 15 available isolates of *Ilyonectria* -like species that had been isolated from kiwifruit were identified using the currently recommended, four gene taxonomic system. Those isolates were *I. europaea* (n=10), *I. robusta* (n=2) and *Ilyonectria* sp. nov. (n=3). In objective 4 (*To increase knowledge of the species associated with root rot in kiwifruit*) the culture collection was increased by an additional 48 isolates to 63 from a survey of vines and ad-hoc testing. The additional isolates were identified as *I. liriodendri* (n=19), *I. robusta* (n=18), I*. europaea* (n=10) and *I. torresensis* (n=1). Thus, a total of five *Ilyonectria* species have been recovered from kiwifruit in New Zealand (*Ilyonectria europaea, I. liriodendri, I. robusta, I. torresensis* and *Ilyonectria* species nov.); of these *I. europaea, I. liriodendri* and *I. robusta* were predominant, comprising 94% of the cultures (Figure 1). Two of these species, namely *Ilyonectria europaea* and *I. liriodendri* have also been shown to be pathogenic towards grapevines (Cabral et al. 2012). In the final

set of objectives for this project (2 and 3) we determine whether the predominant species are pathogenic to kiwifruit, and if isolates of those same species recovered from grapevines are also pathogenic towards kiwifruit.

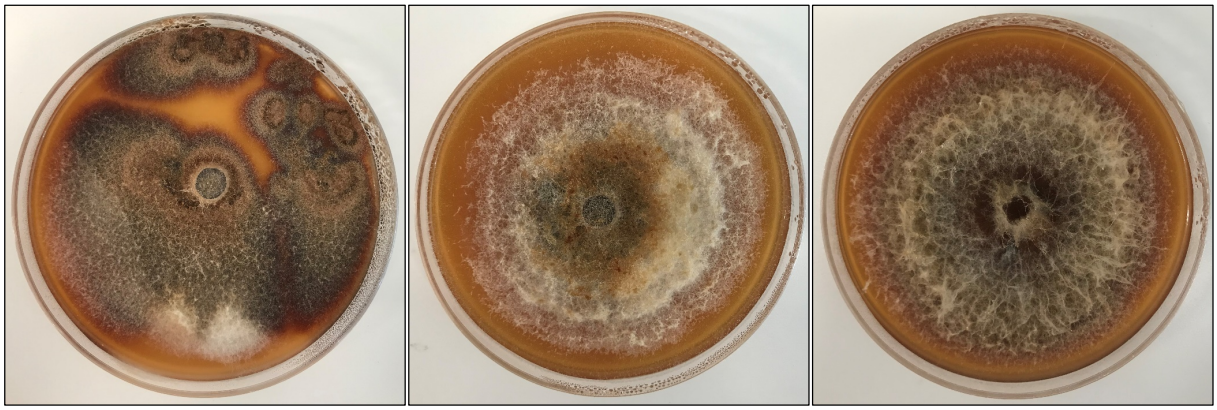

Figure 1. Left to right: Cultures of *Ilyonectria europaea*, *I. liriodendri* and *I. robusta*.

2 Methods

A series of three pathogenicity tests was set up on 21 September (set 1), 28 September (set 2) and 5 October 2020 (set 3). The method of pathogenicity testing used in this study was adapted from Pathrose et al. (2010), who devised a method for investigating the pathogenicity of *Ilyonectria* spp. in the roots of grapevines.

2.1 Fungal isolates

A total of 15 isolates of *Ilyonectria* and *Dactylonectria* were tested for their pathogenicity towards kiwifruit. These isolates consisted of three isolates each of *Ilyonectria europaea*, *I. liriodendri* and *I. robusta* from *Actinidia* sp. (kiwifruit), and two isolates each of *I. europaea*, *I. liriodendri* and *Dactylonectria macrodidyma* from *Vitis* sp. (grapevine) (Table 3). *Dactylonectria macrodidyma* is known to be highly pathogenic on *Vitis* sp. Each isolate had originally been isolated from the trunks or roots of *Actinidia* sp. or *Vitis* sp., stored under standard conditions and was revived for experiments by growing on malt extract agar (MEA) for 7 days at 20°C before use.

2.2 Plant material

Tissue-cultured plantlets of *Actinidia chinensis* var. *deliciosa* 'Hayward' were potted into 8-cm diameter planting bags and grown in a glasshouse (average temperature of 25 ± 5°C). Plants were fed liquid fertiliser periodically following the manufacturer's instructions and kept at a height of approximately 40 cm. After 12-18 months, each plant was carefully uprooted and roots were washed with sterile water. Young outer feeder roots were removed. For consistency and ease of visualising expression of symptoms, roots of similar diameter and coloration were selected for use in the assay, and cut to lengths of c. 8 cm. Roots were mildly surface-sterilised before use by immersion in 0.35% NaOCl for 1 min, then rinsed twice in sterile reverse-osmosis (RO) water.

2.3 Pathogenicity assay

This assay investigated the pathogenicity of the isolates towards the roots of kiwifruit. It was repeated three times. For the first pathogenicity test (set 1), 10 replicate roots per fungal isolate were placed individually into Petri dishes containing 1.5 tbsp. sterile vermiculite, wetted with 9 mL sterile RO water. Fine feeder roots and c. 8 cm of the adjoining root were used. The basal cut end of each root was inserted through a hole drilled into the cap of a 1.7 mL tube filled with sterile RO water to prevent dehydration of the root. The apical end of each root was cut across to create a fresh wound, and a mycelial plug from the growing edge of a 7-day-old culture was placed over the cut end. Sterile MEA plugs served as controls. For the subsequent pathogenicity tests (sets 2 and 3), the procedure remained largely the same, but the Petri plates were lined with thick filter paper wetted with 7 mL sterile RO water, and the chosen roots were slightly thicker (1-1.5 mm in width).

Table 3. *Ilyonectria* group isolates from kiwifruit and grapevine used in the pathogenicity testing

Species	Collection #	Original number	Host	Location
Ilyonectria europaea	cc962	2335.1.6	*Actinidia* sp.	Bay of Plenty, Pyes Pa
Ilyonectria europaea	cc966	2250.2.7a	*Actinidia* sp.	Edgecumbe
Ilyonectria europaea	cc1188	3101.17.2F	*Actinidia* sp.	Motueka
Ilyonectria liriodendri	cc1040	K7	*Actinidia* sp.	Motueka
Ilyonectria liriodendri	cc1190	3101.13.1D	*Actinidia* sp.	Motueka
Ilyonectria liriodendri	cc1191	3101.14.4.3A	*Actinidia* sp.	Motueka
Ilyonectria robusta	cc965	2337.2.6	*Actinidia* sp.	Bay of Plenty, Pyes Pa
Ilyonectria robusta	cc1044	K41	*Actinidia* sp.	Riwaka
Ilyonectria robusta	cc1189	3073.20.3B	*Actinidia* sp.	Paengaroa
Dactylonectria macrodidyma	cc1181	LUPP1006 (Co6a)	*Vitis vinifera*	Central Otago
Dactylonectria macrodidyma	cc1182	LUPP1086 (Mar9b)	*Vitis vinifera*	Marlborough
Ilyonectria europaea	cc1186	LUPP982 (WPa1a)	*Vitis vinifera*	Waipara
Ilyonectria europaea	cc1187	ICMP16794	*Vitis vinifera*	North Canterbury
Ilyonectria liriodendri	cc1183	LUPP959 (Wpa1e)	*Vitis vinifera*	Waipara
Ilyonectria liriodendri	cc1184	LUPP986 (HB2d)	*Vitis vinifera*	Hawkes Bay

2.4 Isolation and assessment

After 21 days' incubation, each root was surface-sterilised by immersion in 70% ETOH for 3 s, 0.35% NaOCl for 3 min, rinsed twice in sterile RO water, then air-dried. Measurements were taken of the entire root piece, and the lesion (blackened area) from the root tip. To confirm infection by the pathogens, recovery of the inoculated fungal isolate was done from the roots. Each root was sequentially cut into 1 cm lengths for 6 cm from the inoculated root tip, with each length placed on PDA amended with antibiotics and re-identified by morphological techniques after 7-14 d.

2.5 Statistical analysis

Data from each experiment were analysed separately, because the responses to the various inoculation treatments varied between the three trials. Lesion lengths were analysed with analysis of variance. Recovery of the inoculated fungal isolate (treatment) was analysed in two ways. The percentage of roots that formed colonies (positions > 0) were analysed with a Bernoulli generalised linear model (GLM, McCullagh and Nelder 1989), with a logit link. For each analysis, various contrasts between the hosts, species and isolates were assessed, with F-tests for analysis of variance, and with X^2 tests within the analyses of deviance for the analyses of the Colony positions. Mean Lengths and %with colonies are presented 95% confidence limits. For the % with colonies, there were obtained on the logit scale, and converted to the percentage scale. All analyses were carried out with Genstat (Payne et al. 2019).

3 Results

3.1 Development of root lesions

Actinidia sp. roots were inoculated with fungal pathogens that had originally derived from either *Actinidia* sp. or *Vitis* sp. Discolouration (browning) of the root from the apical end was termed 'lesion' and was measured for each root piece. Lesions were present in replicates of the control (uninoculated) and inoculated (plus pathogen) root pieces for all three experiments. Lesion length varied from 0 to 13, 13 and 8 cm for each of the three experiments, respectively, with means of 5.1 cm (thinner roots), 2.2 cm and 2.8 cm, respectively. Note – roots selected for the first experiment were from the youngest plants and were thinner than those used in experiments 2 and 3. Overall, lesion length was significantly different between the isolates for each of the three experiments (p=0.025, p=0.007 and p=0.004 for an overall test, respectively) (Figure 2).

For experiment 1, the major difference (p=0.005) was between the uninoculated control (mean=2.9 cm) and the mean for the pathogen inoculated roots (5.3 cm). Lesions were on average significantly longer for roots inoculated with pathogens originally derived from *Actinidia sp.* (mean=5.6 cm) than for those derived from *Vitis* sp. (mean=4.8 cm; p=0.041). There was no significant difference between the lesion lengths produced by pathogens originally derived from the same host species. With *Vitis* sp. means of 5.6 cm, 4.0 cm and 5.2 cm for *D. macrodidyma*, *I. europaea*, *I. liriodendri* respectively (p=0.200); *Actinidia* sp. means of 4.8 cm, 5.9 cm and 5.5 cm for *I. europaea*, *I. liriodendri* and *I. robusta* respectively, (p=0.775). In addition, lengths for roots inoculated with the different isolates of the same pathogen, originally isolated from a given host, did not differ significantly (p=0.052 and p=0.996 for isolates originally from *Vitis* sp. and *Actinidia* sp., respectively).

For experiment 2, there was little difference between the uninoculated control (mean=1.4 cm) and the mean of roots inoculated with the fungal pathogens (mean=2.3 cm; p=0.200). This was similar for experiment 3 where there was no significant difference between the uninoculated control (mean=2.5 cm) and the pathogen inoculated roots (mean=2.8 cm, p=0.065). For both trials, on average, lesion lengths did not vary according to the host they were derived from. For experiment 2, the mean lesion lengths were 2.0 cm for pathogens originally isolated from *Vitis sp*. and 2.5 cm for those from *Actinidia sp.* (p=0.126), and for experiment 3, 2.5 cm for those recovered from *Vitis sp.*, and 3.1 cm for those from *Actinidia sp.* (p=0.119)

For experiments 2 and 3, mean lesion lengths for roots inoculated with isolates originally derived from *Vitis sp.* did not vary (Experiment 2 cm, 2.5 cm, 2.0 cm, 2.2 cm, p=0.798 and Experiment 3 cm, 3.1 cm, 2.7 cm and 3.1 cm; p=0.123 for *D. macrodidyma*, *I. europaea*, *I. liriodendri*, respectively). The lesions produced by the isolates within a pathogen species recovered from a particular host (*Vitis* sp. vs *Actinidia* sp.) were not significantly different (p=0.25 and 0.92 for *Vitis* sp. and *Actinidia* sp. respectively), except for *D. macrodidyma* in experiment 3 where the lesion length was greater for cc1181 (3.8 cm) than for cc1182 (1.6 cm; p=0.031).

3.2 Recovery of the pathogen from infected roots

For experiment 1, the inoculated species were not recovered from the control roots. The fungal pathogens were recovered from all inoculated roots, for all replicates (100%) (Figure 2). This pattern was repeated for the other two experiments, although the recovery rate for some pathogen species was less than 100%, but in all cases was over 80% (Figure 2).

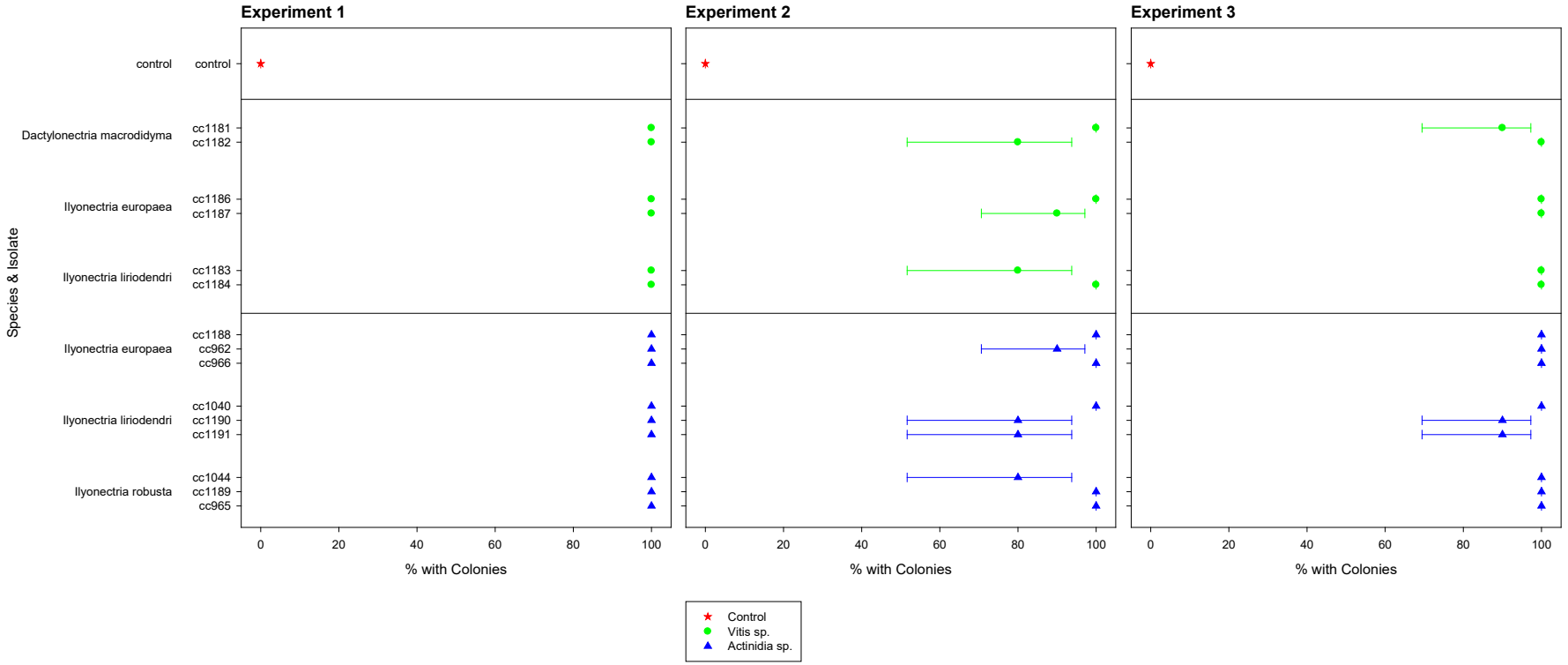

Figure 2. Percentage colony recovery for each pathogen species and isolate from inoculated kiwifruit roots, for the three experiments. Error bars are 95% Confidence limits. Note that the upper limit for a mean of 0 and the lower limit for a mean of 100 are difficult to obtain, so are not shown.

3.3 Colonisation of the roots by the fungal pathogens (endophytic movement)

The median position to which each fungal pathogen colonised the kiwifruit roots is summarised in Table 4. The test fungi were not isolated from control roots. All fungal pathogens colonised the roots with some variation between pathogen species and isolates.

Table 4. Median length (cm from inoculation point) colonised by fungal pathogens originally isolated from either *Vitis* sp. or *Actinidia* sp. in detached kiwifruit roots, in each of the three experiments

Plant host that pathogens were originally isolated from	Pathogen species	Pathogen isolate number	Colonised length (cm) in each experiment		
			1	2	3
Control	uninoculated	-	0.0	0.0	0.0
Vitis sp.	*Dactylonectria macrodidyma*	cc1181	6.0	6.0	5.0
		cc1182	5.0	3.5	5.0
	Ilyonectria europaea	cc1186	4.5	6.0	5.0
		cc1187	6.0	5.0	6.0
	Ilyonectria liriodendri	cc1183	6.0	4.5	6.0
		cc1184	5.0	6.0	6.0
Actinidia sp.	*Ilyonectria europaea*	cc1188	6.0	5.0	5.0
		cc962	5.0	4.0	5.0
		cc966	6.0	6.0	5.0
	Ilyonectria liriodendri	cc1040	6.0	6.0	5.0
		cc1190	6.0	3.0	5.5
		cc1191	6.0	5.0	6.0
	Ilyonectria robusta	cc1044	4.5	5.0	5.0
		cc1189	6.0	6.0	6.0
		cc965	6.0	5.5	5.0

The ability of the fungal pathogens to move within the roots varied between the isolates ($p<0.001$ for all three experiments). The main difference was between the uninoculated control and the median of the inoculated root pieces (plus pathogen) ($p<0.001$ for all experiments). When variation between pathogen species and the isolates within a pathogen species was assessed, there were few differences (most $p>0.05$). The exceptions were for experiment 2, where there was variation between the pathogen species originally isolated from *Vitis* sp. ($p=0.020$) and for isolates initially recovered from *Actinidia* sp.

4 Discussion

The aim of this research was to determine whether isolates of the main 'Cylindrocarpon-like' species recovered from the kiwifruit survey in BS1932 (*Ilyonectria* sp.) were able to infect kiwifruit (*Actinidia* sp.) roots, and if isolates of those same species (*Ilyonectria* sp. and one *Dactylonectria* sp.) recovered from grapevines (*Vitis* sp.) were also able to infect kiwifruit roots.

Overall the data showed that inoculation with the *Ilyonectria/Dactylonectria* sp. produced larger lesions in the inoculated compared to control roots. No colonisation was found in the uninoculated roots, suggesting that the discolouration of these roots was a general response to wounding and experimental duration. In contrast, larger lesions (approx. 2 × larger) were produced in roots inoculated with the fungal pathogens and all of the pathogens were able to be recovered as endophytic colonisers of the root tissue, irrespective of whether they had originally been isolated from *Actinidia* sp. or *Vitis* sp. These results were obtained using a newly developed detached kiwifruit assay which provided a rapid method for assessing the pathogenicity of fungal isolates. This methodology may prove useful for other researchers on root pathogens of kiwifruit and we anticipate publishing it in New Zealand Plant protection.

This work was comprehensive, testing 15 isolates from four pathogen species in three separate experiments. All experiments were congruent showing that there was greater lesion (discolouration) development in inoculated roots. Discolouration and rotting of roots are typical infection symptoms of *Ilyonectria* and *Dactylonectria* sp. and similar symptoms were produced on the roots of *Vitis* sp. in the experiments of Pathrose et al (2010). Thus, the isolates from kiwifruit demonstrate infectivity and pathogenicity that is typical of the genera and has been observed on the root tissue of other hosts.

This work also showed that species/isolates of *Ilyonectria* recovered from *Vitis* sp. were able to infect the roots of kiwifruit. Members of the *Ilyonectria/Dactylonectria* genera are well-known causal agents of root rots in a number of host plants including grape (Probst et al. 2019), avocado (Parkinson et al. 2019), ginseng (Zhang et al. 2019) and berryfruit (strawberry, raspberry, blackberry) (Sanchez et al. 2019). Cross-infection and virulence of species on multiple hosts has been previously demonstrated (Cabral et al. 2012). The data presented here suggest that the species of these genera present on other hosts may present an inoculum reservoir for kiwifruit. Some species are known to produce long-lived chlamydospores in soil (Chaverri et al. 2011); as infective propagules this may be a problem for new orchards that are placed on sites previously planted with other fruiting host species.

The presence of vine decline and trunk disease is an ongoing problem for the kiwifruit industry since it was first identified in the early 2000s. The data presented here indicate that members of the *Ilyonectria* genus may contribute to vine decline. Currently it is unknown whether the *Ilyonectria* group is a causal or associative pathogen in terms of kiwifruit root rots and KTDs. It is, however, likely part of a complex of fungi involved in these diseases. Future research should focus on the ability of the three main *Ilyonectria* species, either alone or in complexes, to develop symptomology on small plants. As *Ilyonectria* spp. tend to be soil-borne organisms, infection capability against different kiwifruit rootstocks (e.g. 'Bruno', 'Kaimai') should also be studied. As KTDs become more apparent in orchards, growers are removing the most affected vines. Even with removal of the greater parts of the vine, this is likely to leave large pieces of root underground, likely forming a reservoir of inoculum. The length of time that these roots remain a significant source of inoculum would inform risk for re-planting.

5 Acknowledgements

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