Fate of mycelial and conidial propagules of *Ilyonectria* and *Dactylonectria* species in soil

Chantal M. Probst[#], Dudley Crabbe, Hayley J. Ridgway[†], Marlene V. Jaspers and E. Eirian Jones*

Department of Pest-management and Conservation, Lincoln University, PO Box 5084, Lincoln 7647, Canterbury, New Zealand
[#]Current address: Biodiversity and Conservation Group, Manaaki Whenua - Landcare Research, Auckland 1072, New Zealand
[†]Current address: The New Zealand Institute for Plant and Food Research Ltd, Christchurch 8140, New Zealand
**Corresponding Author: Eirian.Jones@lincoln.ac.nz*

Abstract Black foot disease of grapevines causes significant economic loss to the viticulture industry worldwide. A novel method was developed to investigate the fate of propagules of three species associated with black foot disease in New Zealand, *Dactylonectria macrodidyma, Ilyonectria europaea* and *I. liriodendri*, in soil. Conidia or mycelium of one isolate each of the three species were buried in soil in nylon mesh bags, and conidia/chlamydospore numbers were determined microscopically after 2 and 3 weeks. Conidia and chlamydospores were produced by mycelial inocula of all isolates, with greater numbers of chlamydospores after 3 weeks. Conidial inocula of all isolates also produced chlamydospores. Chlamydospores were formed at either the terminus or side of a hypha, and single and multiple conidia formed chlamydospores by combining their cellular protoplasm. Chlamydospores were produced from conidia, and conidia from mycelium faster for the *I. europaea* isolate than the *D. macrodidyma* and *I. liriodendri* isolates. The rapid formation of chlamydospores as survival propagules will facilitate the ability of these pathogens to persist in soil in the absence of a host.

Keywords *Cylindrocarpon* spp., *Cylindrocarpon destructans, C. macrodidymum, C. liriodendri*, blackfoot disease, grapevines

INTRODUCTION

Black foot disease is a major economic problem in grapevine nurseries and vineyards worldwide, with infected vines often being stunted with reduced vigour, shortened internodes and sparse chlorotic foliage (Halleen et al. 2006). Below ground, black, sunken, necrotic lesions develop on the roots and the wood tissue at the base of the trunk develops a reddish brown to dark purplish discolouration, which eventually spreads across the whole trunk, ultimately often resulting in the death of vines. The causal agents include species belonging to a number of different genera such as *Cylindrocarpon, Cylindrocladiella, Ilyonectria* and *Dactylonectria* species (Cabral et al. 2012a, b; Brown et al. 2013; Agustí-Brisach & Armengol 2013; Lombard et al. 2014; Úrbez-Torres et al. 2014). The recent taxonomic revision of *Cylindrocarpon* spp. has resulted in the reclassification of the species associated with black foot disease as either the genus *Dactylonectria* or *Ilyonectria*, with several new species defined within each (Cabral et al. 2012a, b; Lombard et al. 2014). Based on the new nomenclature, a survey of declining vines from the main grape-growing regions in New Zealand identified *Ilyonectria liriodendri, I. europaea* and *Dactylonectria macrodidyma* as being the main

species associated with the disease complex (Pathrose 2012; Pathrose et al. 2014; Outram et al. 2014).

Black foot pathogens are reported to have similar disease cycles, with grapevine roots and stem bases infected by pathogen propagules or infected host debris remaining in the soil after the removal of infected plants (Agustí-Brisach & Armengol 2013). Recent work has shown that all propagules (chlamydospores, conidia and mycelium colonised organic material) of *I. liriodendri* and *D. macrodidyma* are able to infect grapevine rootstocks when inoculated and planted in potting mix (Probst et al. 2019). However, mycelial inoculum is likely to be transient in soil in the absence of a suitable substrate. Overall, little is known about the epidemiology of these propagules in soil including their survival in soil and whether they are recoverable or rapidly disintegrate, and if vegetative mycelium and asexual conidia convert into 'survival' structures such as chlamydospores over time. Previous studies have suggested that for *I. radicicola*–complex (as *C. radiciola*) and *Fusarium oxysporum*, conidia maybe involved as a distribution propagule, but in the absence of a suitable substrate for growth, such as a host, they either convert into chlamydospores or disintegrate (Taylor 1964). However, whether the different species associated with black foot disease of grapevines vary in respect to their ability to convert into resistant chlamydospores in the absence of a host and, therefore, to survive is not known. The aim of the research was to use a novel method developed in the study to determine the fate of mycelial and conidial propagules of *I. liriodendri, I. europaea* and *D. macrodidyma* in soil providing information as to the survival of inocula in soil. The results of the study will contribute to the understanding of the epidemiology of black foot pathogens and provide information as to the likely risk of infection of vines replanted into vineyard or nursery sites after infected plants have been removed.

MATERIALS AND METHODS

Conidia

For this experiment, one isolate each of *D. macrodidyma* Gis3d (ICMP 16789), *I. europaea* WPala (ICMP16794) and *I. liriodendri* Mar19f (ICMP16793) were selected from the Lincoln University Plant Pathology culture collection. These isolates were grown on potato dextrose agar (PDA; Oxoid Ltd, Basingstoke, UK) and conidia were harvested from the plates by flooding 3-week-old cultures with sterile water containing 0.01% v/v Tween 80 and scrapping the surface with a sterile glass microscope slide. The resulting suspension was sieved through a 150-µm sieve to remove mycelial fragments and then centrifuged at 3400 × g for 10 min. The pellet was suspended in 1 mL of sterile water and the concentrations of the conidial suspensions adjusted to 10^8 conidia/mL based on haemocytometer counts. Bags (90 × 85 mm) were made of nylon mesh (20-µm pore size; Schweizer Seidengaze-fabrik AG, Thal, Switzerland) with edges that were heat-sealed. After placing 15 g of autoclaved fine dry silica sand (particle size 50–500 µm) and 1 mL of a spore suspension into each bag, the sand was wetted with approximatively 8 mL of sterile water and the bags were heat-sealed. The bags were then buried in a 50:50 mixture of soil (sieved through 4-mm mesh) and potting mix (80% horticultural bark (grade 2) and 20% pumice) in 4-L plastic square containers (170 × 170 × 80 mm) at a depth of 20 mm.

Mycelium

For each isolate, mycelium was produced in liquid culture. A mycelial plug (5.5-mm diameter) cut from the edge of a 14-day-old PDA colony was placed in 100 mL of autoclaved potato dextrose broth (PDB; Difco laboratories, Becton Dickinson, USA) contained in a 250-mL conical flask. The flasks were incubated on a shaker at 100 rev/min at room temperature for 4 days. The mycelia were then harvested before the formation of spores. For each flask, the contents were poured into a mesh bag via a funnel with the waste collected in a beaker underneath. The agar plugs were then removed and each bag rinsed

with approximatively 10 mL of sterile water to remove any PDB, before being placed onto sterile paper towels to remove any excess liquid. Each bag was then heat sealed and buried into the same soil container as used for a bag of conidia.

Assessment

There were six replicates (blocks) for each propagule and isolate. The experiment was laid out in a randomised block design with two blocks being randomly selected for examination after 2 weeks in the soil and the remaining four blocks were harvested after 3 weeks. The content of each bag was emptied into a Petri dish and the bag was rinsed with 5 mL of sterile water containing 0.01% Tween 80 and this was added to the Petri dish. The different structures observed in the bags were recorded after 2 and 3 weeks. The numbers of conidia and chlamydospores were determined after 3 weeks based on haemocytometer counts. The data were $\log_{10}$ transformed prior to analysis using ANOVA with Genstat version 16 (VSN International Ltd, Hemel Hempstead, UK) with treatment means compared using Fisher's Protected least significant difference (LSD) test at P=0.05.

RESULTS

Observation of mycelium and conidium in bags after 2 and 3 weeks in the soil showed the presence of conidia, chlamydospores and mycelium (Table 1). The conversion of conidia and mycelium into chlamydospores followed different patterns. The basic chlamydospore structure was a single melanised spherical cell. Chlamydospores originating from mycelium were formed on the side (Fig. 1A-a) or at the terminal end of a hypha (Fig. 1A-b), alone or in a chain, that generally comprised three to five cells (Fig. 1B).

A single conidium formed one or two adjacent chlamydospores. The latter was formed when the contents of a four-celled conidium moved to two cells on one extremity of the conidium (occasionally the contents moved to the middle), these cells then started to swell and to form melanised walls, resembling the figure eight (Fig. 2). Chlamydospores in clusters appeared to be either an aggregation of single chlamydospores or the formation of multiple single chlamydospores in a group, and were only found in conidial bags for the different species.

Occasionally, a conidium formed a short

Table 1 Structures found in conidial (con.) and mycelial (myc.) bags from *Dactylonectria macrodidyma, Ilyonectria europaea* or *I. liriodendri* isolates, 2 and 3 weeks after being buried in soil (+ : structure present in week 2 and 3, - : structure absent, a: structure present in week 2 and b: structure present in week 3).

	D. macrodidyma		*I. europaea*		*I. liriodendri*	
Structures	Con.	Myc.	Con.	Myc.	Con.	Myc.
Conidia	+	a	+	+	+	+
Germinated conidia	+	-	+	-	+	b
Empty conidia	+	-	-	-	+	-
Conidia forming chlamydospores	b	a	-	-	+	a
Conidia formed chlamydospores	+	-	-	-	+	-
Single chlamydospores	-	+	b	+	+	+
Clusters of chlamydospores	+	a	+	-	+	+
Chains of chlamydospores	-	-	-	+	-	a
Chlamydospore at terminal end of germ tube	+	+	b	+	b	-
Pair of chlamydospores	a	-	+	+	+	+

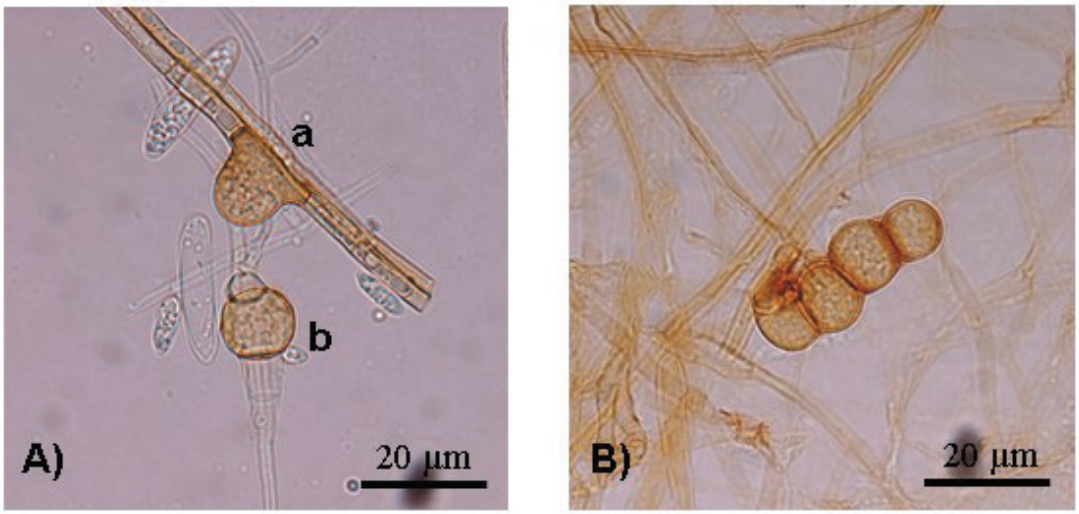

Figure 1 Single chlamydospores formed on (A) the side (a) or at the terminal end (b) of a hypha; (B) chains of chlamydospores formed from mycelial inoculum incubated in soil. Scale bar represents 20 µm.

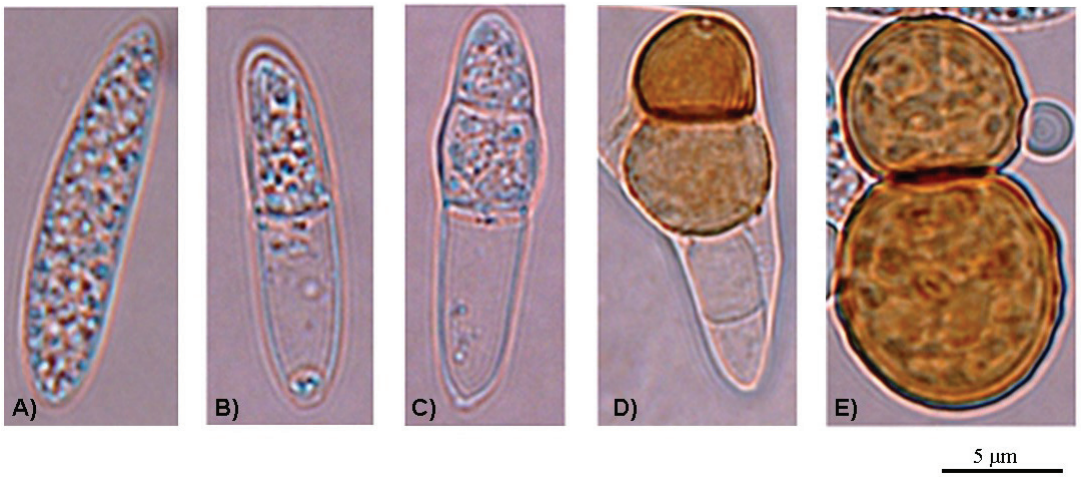

Figure 2 Conversion of a conidium into two chlamydospores. (A) conidium; (B) contents of conidium moving to one extremity; (C) cells of conidium swelling; (D) melanisation of the two cells; (E) formation of two chlamydospores. Scale bar represents 5 µm.

germ tube that fused to another conidium and the contents of one conidium were transferred to the other conidium that then formed multiple chlamydospores (Figs. 3A & 3B). The same phenomenon was observed to occur between multiple conidia (Figs. 3C & 3D).

For *I. europaea*, the bags filled with conidia were found to contain a mixture of chlamydospores and conidia (some germinated) after 2 and 3 weeks. The chlamydospores were present in pairs or clusters. Single chlamydospores and/or chlamydospores at the terminal end of a germ tube were observed after 3 weeks only (Table 1). The bags filled with mycelia were found to contain conidia and chlamydospores after 2 and 3 weeks. Chlamydospores were observed: alone;

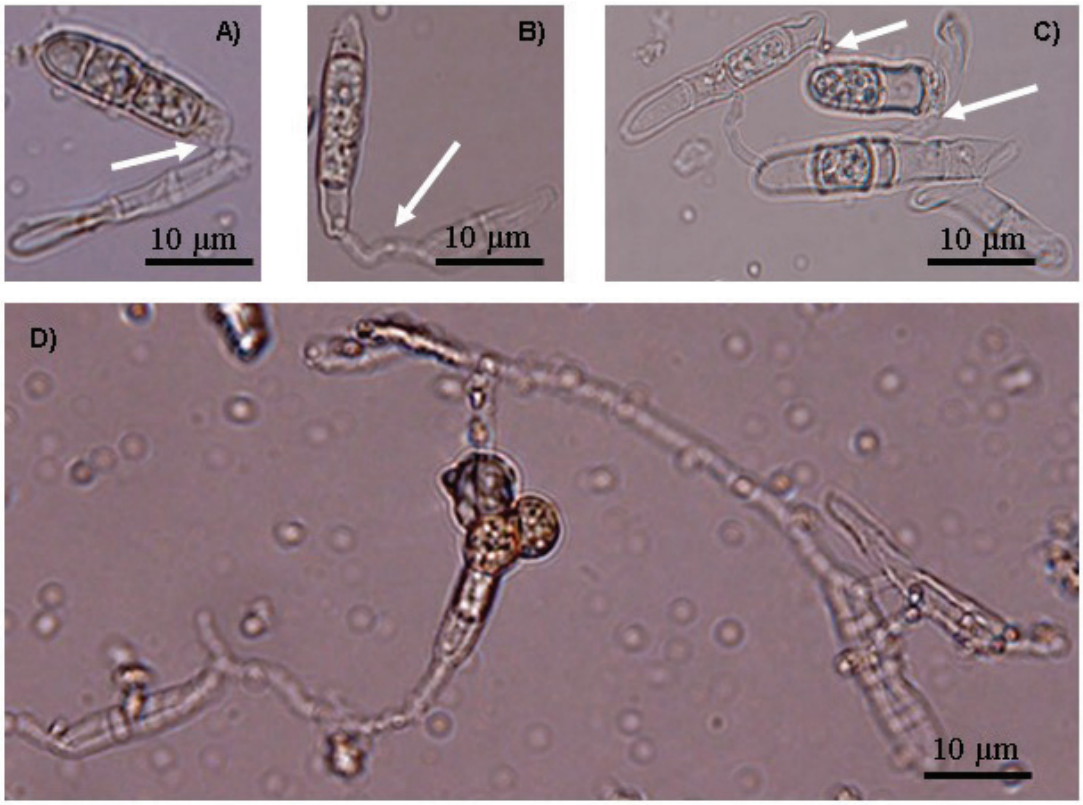

Figure 3 Conidia attached with germ tubes (arrow) and chlamydospore formation. Two conidia attached with (A) chlamydospore formation on extremity of conidium; and (B) chlamydospore formation in the middle of conidia. Multiple conidia attached with (C) formation of single chlamydospores; and (D) formation of cluster of chlamydospores. Scale bar represents 10 µm.

in chains; in pairs; or at the terminal end of a germ tube. They were formed at the terminal end of a hypha or on the side of a hypha. Conidium germination was not observed in the mycelial bags (Table 1).

For *I. liriodendri*, the bags initially containing conidia were found to contain a mixture of chlamydospores and conidia after 2 and 3 weeks. Chlamydospores occurred singly, in clusters and/or in pairs with conidia. Empty conidia and conidia in the process of forming and having formed chlamydospores were also observed. Chlamydospores at the terminal end of a germ tube were observed after 3 weeks only (Table 1). The bags originally containing mycelia were found to contain conidia and chlamydospores (singly and in pairs or clusters) after 2 and 3 weeks. Conidia forming chlamydospores and chains of chlamydospores were observed at week 2 while germinating conidia were observed in week 3 (Table 1).

For *D. macrodidyma*, pairs of chlamydospores were observed in the bags initially containing conidia in week 2. Germinating conidia, empty conidia, conidia formed chlamydospores, clusters of chlamydospores and chlamydospores at the terminal end of germ tubes were all observed after 2 and 3 weeks while conidia forming chlamydospores were observed in week 3 only (Table 1). The bags filled with mycelia

were found to contain single chlamydospores and chlamydospores at the terminal end of germ tubes after 2 and 3 weeks. Conidia forming chlamydospores and chlamydospore clusters were observed in week 2 only (Table 1).

After 3 weeks, there was a significant interaction between inoculum type (mycelium or conidia) and species on both the number of conidia and chlamydospores recovered ($P<0.001$ for both). The mean number of conidia remaining in the bags originally filled with conidia were significantly lower for *I. europaea* (5.54 $\log_{10}$ conidia) than *D. macrodidyma* (6.60 $\log_{10}$ conidia), with *I. liriodendri* (6.04 $\log_{10}$ conidia) not being significantly different to either *I. europaea* or *D. macrodidyma* (Table 2). The mean number of chlamydospores was significantly higher in bags of conidia after 3 weeks for *I. liriodendri* (5.78 $\log_{10}$ chlamydospores) than *I. europaea* (4.83 $\log_{10}$ chlamydospores), with *D. macrodidyma* (5.36 $\log_{10}$ chlamydospores) not being significantly different to either *I. europaea* or *I. liriodendri*. For the bags originally filled with mycelia, no significant differences were observed for the presence of conidia and chlamydospores for the different species.

DISCUSSION

Mycelium and conidia from *Dactylonectria macrodidyma, Ilyonectria europaea* and *I. liriodendri* that were incubated in a soil environment were shown in this study to produce chlamydospores, thus enabling these species to persist in the soil for 3 weeks in the absence of the host plant, grape. Chlamydospores were formed either at the terminal end, or on the side, of a hypha, and either single or multiple conidia formed chlamydospores within their cells by combining their protoplasm. These observations were similar to those of Matturi and Stenton (1964) who reported that conidia of *Cylindrocarpon* spp. incubated in soil formed chlamydospores after 3 days of incubation. As in the current study, the chlamydospores were observed to form from hypha, or conidia where one to two cells formed resistant walls. Taylor (1964) reported that most *I. radicicola*–complex (as *C. radicicola*) conidia did not germinate in acid and alkaline soils, but a few formed chlamydospores within them, while germinated conidia developed short germ tubes that formed chlamydospores. Similar observations were made with *Neocosmospora phaseoli* (syn. *Fusarium solani* f. sp. *phaseoli*) (Nash et al. 1961). The observation in the current study that chlamydospores often resulted from

Table 2 Conidium and chlamydospore numbers ($\log_{10}$) present in *Dactylonectria macrodidyma*, *Ilyonectria europaea* or *I. liriodendri* conidial and mycelial bags, 3 weeks after being buried in the soil. Initially (week 0), conidial bags contained 8.0 $\log_{10}$ (10^8) conidia and no chlamydospores, mycelial bags contained no conidia and chlamydospores.

Isolate	Inoculum	Conidia	Chlamydospores
D. macrodidyma	Conidia	6.60 a[1]	5.36 ab[1]
	Mycelium	4.85 cd	5.28 ab
I. europaea	Conidia	5.54 bc	4.83 b
	Mycelium	5.23 cd	5.32 ab
I. liriodendri	Conidia	6.04 ab	5.78 a
	Mycelium	4.69 d	4.94 b
LSD		0.74	0.57

[1]Mean values in each column followed by different letters are significantly different based on LSD at $P\leq0.05$.

the transfer of protoplasm from one conidium to a second conidium linked by germ tubes is similar to that reported by Matturi and Stenton (1964). This finding indicates that, in many cases, a single conidium was not able to convert into a chlamydospore, with the cellular contents of more than one conidium required for the formation of chlamydospores.

In this study, chlamydospores were not distinguished by their origin, as conidia were produced in bags originally containing mycelia and formed chlamydospores. However, Matturi and Stenton (1964) recorded the number of chlamydospores originating from conidia and hyphae and found that chlamydospores of the *I. radicicola*–complex (as *C. radicicola*) were mostly formed in conidia in unfavourable conditions, with 32 and 91% of chlamydospores originating from conidia when incubated in soil with pH 7.4 and 3.8, respectively after 12 days. In contrast, chlamydospores were formed from hyphae at higher rates at pH 7.4 compared with pH 3.8.

Different patterns of chlamydospore development were observed among the individual isolates of each species examined. For the single *I. europaea* isolate studied here, the transformation of conidia into chlamydospores occurs shortly after conidia were placed in the bags as no intermediate stages of chlamydospore formation were observed at 2 weeks. However, for both *I. liriodendri* and *D. macrodidyma* isolates, different stages of chlamydospore conversion were present in the bags originally filled with conidia, which indicates that the transformation occurred either later or progressively. In the bags filled with mycelia, the single *I. europaea* isolate studied also produced conidia faster than the *D. macrodidyma* and *I. liriodendri* isolates. The presence of dumb-bell shaped chlamydospores indicates an early transformation of conidia into chlamydospores. The *I. liriodendri* isolate exhibited most stages of chlamydospore formation, while only early stages of chlamydospore formation were observed for the *D. macrodidyma* isolate suggesting that *D. macrodidyma* produce chlamydospores more slowly than the *I. europaea* and *I. liriodendri* isolates. Matturi and Stenton (1964) also reported differences in the rate of chlamydospore formation in different '*Cylindrocarpon*' species, with *I. radicicola*–complex (as *C. radicicola*) producing chlamydospores more rapidly compared with three other former '*Cylindrocarpon*' species (*Thelonectria discophora* (as *Cylindrocarpon janthothele* var. *minus*), *Neonectria punicea* (as *C. album*) and *Nectria ramulariae* (as *C. ehrenbergii*)). The rapid rate of conversion of thin-walled hyphae and conidia into thick-walled survival chlamydospores may indicate the potential for better survival of *I. europaea* and *I. liriodendri* inocula in soil. However, whether different isolates within these species vary in their propagule conversion rates needs further study.

After 3 weeks, the number of conidia in all the bags originally filled with conidia had declined but there were significantly lower numbers of *I. europaea* conidia than for *D. macrodidyma* and *I. liriodendri*. The reduction of the number of conidia was 286–, 91– and 25–fold for *I. europaea, D. macrodidyma* and *I. liriodendri*, respectively. The single *Ilyonectria liriodendri* isolate studied here produced chlamydospores earlier or faster than the *D. macrodidyma* isolate studied as *I. liriodendri* had a higher number of chlamydospores and a lower number of conidia than the latter. Similarly, Matturi and Stenton (1964) observed a 70– and 280–fold reduction in the number of *I. radicicola*–complex (as *C. radicicola*) conidia in soils at pH 7.4 and 3.8, respectively after 12 days while the decrease was lower for other species. They associated this reduction and the loss of mycelium to the activity of bacteria and undetermined lysis, and concluded that mycelium and conidia are transient in soil and are rapidly lysed by microorganisms that activate chlamydospore formation. The slower conversion of conidia into chlamydospores by the *D. macrodidyma* isolate studied here as discussed earlier may result in reduced survival of this isolate in soil. The chlamydospores are reported to remain dormant in soil without organic matter until they are stimulated by plants or destroyed by other microorganisms (Matturi & Stenton 1964; Taylor

1964). Taylor (1964) observed that, in natural soil, hyphal development was restricted and conidia and mycelium were both absent after 1 month, while chlamydospores were present.

Another factor affecting the reduction in the number of conidia is the formation of chlamydospores that results from the combination of one to multiple conidial protoplasm, leaving empty cells. Chlamydospore formation was significantly higher for the *I. liriodendri* isolate than *D. macrodidyma* and *I. europaea* isolates studied, which had the lowest number after 3 weeks. The low number of chlamydospores produced by the *I. europaea* isolate might be explained by the fact that the isolate produced mainly one- to two-celled conidia while the *I. liriodendri* and *D. macrodidyma* isolates formed mainly four-celled conidia. This observation indicated that a higher cell number was required to form chlamydospores in these two species (Fig. 3) but further work on different isolates is needed to confirm this.

Chains of chlamydospores were observed only in bags filled with mycelia and only those containing either *I. europaea* or *I. liriodendri*. This situation reflects previous observations on media where chlamydospores were abundant within 14 days for the *I. radicicola*–complex (as *C. destructans*) common for *I. liriodendri* (as *C. liriodendri*) and rare for the *D. macrodidyma*–complex (as *C. macrodidymum*) (Halleen et al. 2004; Halleen et al. 2006).

The current study was conducted to determine the fate of mycelium and conidia of *Dactylonectria* and *Ilyonectria* spp. in soil in the absence of a host plant. Results showed a rapid conversion from active (mycelium and conidia) to survival (chlamydospores) propagules. The influence of a plant host on these processes is unknown and warrants further studies. Probst et al. (2019) showed that an inoculum of the black foot pathogens *D. macrodidyma* and *I. liriodendri* produced from mycelial-colonised barley grain resulted in infection of grapevine rootstocks. However, results from the current study suggest that mycelial inoculum is transient and undergoes lysis in soil or converts into chlamydospores with conidia being an intermediate stage. Whether mycelia directly infect grapevine roots in the soil environment or convert into conidia/chlamydospores that subsequently germinate to infect grapevine roots is not known. Additionally, the fate of mycelium-colonising organic material, rather than as pure mycelial inoculum as used in the current study, is not known. Shorter incubation times could be used to investigate the processes involved and the number of chlamydospores obtained from mycelium in soil as well as from germinated and ungerminated conidia to provide more extensive knowledge of the conversion behaviour of different *Dactylonectria* and *Ilyonectria* spp.

The rapid decline of inoculum concentration of both mycelial and conidial inocula in soil seen in this study is supported by the observations of Probst (2011) where *I. liriodendri* and *D. macrodidyma* soil conidial inoculum levels declined rapidly over time using a quantitative PCR. For *I. liriodendri*, the inoculum levels reduced from 10^5 conidia/g soil to less than 5% (equivalent to 5×10^3 conidia/g soil) after 1 week, but remained constant at this level over a period of 6 weeks, while that of *D. macrodidyma* was reduced below the detection level of 1.9×10^3 conidia/g soil after 6 weeks. Although the inoculum concentration in soil decreases rapidly it is still likely to be sufficient to cause infection, especially where wounded or incompletely callused grapevine planting material is used (Probst et al. 2019). However, how different physiochemical soil factors, such soil types, pH, soil moisture or nutrient availability affect the propagules in soil is unclear and warrants further investigation. The results of the study have shown that the propagules can survive in the soil in the absence of a host, indicating that the risk for infection of vines replanted into vineyard or nursery sites after infected plants have been removed is relatively high. Therefore, knowledge of the factors that influence the survival of these propagules in soil has the potential to inform the development of management practices that reduce to reduce inoculum survival and therefore infection risk.

ACKNOWLEDGEMENTS

The authors acknowledge New Zealand Winegrowers and Lincoln University for funding, and Technology for Industry Funding (Callaghan Innovation), New Zealand Ministry of Science and Innovation for providing a scholarship to the first author.

REFERENCES

Agustí-Brisach C, Armengol J 2013. Black-foot disease of grapevine: and update on taxonomy, epidemiology and management strategies. Phytopathologia Mediterranea 52: 245–261.

Brown DS, Jaspers MV, Ridgway HJ, Jones EE 2013. Susceptibility of grapevine rootstocks to *Cylindrocladiella parva*. New Zealand Plant Protection 66: 249–253.

Cabral A, Groenewald JZ, Rego C, Oliveria H, Crous PW 2012a. Cylindrocarpon root rot: multi-gene analysis reveals novel species within the *Ilyonectria radicicola* species complex. Mycological Progress 11: 655–688.

Cabral A, Rego C, Nascimento T, Oliveria H, Groenewald JZ, Crous PW 2012b. Multi-gene analysis and morphology reveal novel *Ilyonectria* species associated with black foot disease of grapevines (*Vitis* spp.). Fungal Biology 116: 62–80.

Halleen F, Schroers HJ, Groenewold JZ, Crous PW 2004. Novel species of *Cylindroncarpon* (*Neonectria*) and *Campylocarpon* gen. nov. associated with black foot disease of grapevines (*Vitis* spp.). Studies in Mycology 50: 431–455.

Halleen F, Fourie PH, Crous PW 2006. A review of black foot disease of grapevines. Phytopathologia Mediterranea 45: 55-67.

Lombard L, Van der Merwe NA, Groenewald JZ, Crous PW 2014. Lineages in *Nectriaceae*: re-evaluating the generic status of *Ilyonectria* and allied genera. Phytopathologia Mediterranea 53: 515–532.

Matturi ST, Stenton H 1964. The behaviour in soil of spores of four species of *Cylindrocarpon*. Transactions of the British Mycological Society 47: 589–599.

Nash SM, Christou T, Snydeb WC 1961. Existence of *Fusarium solani* f. sp. *phaseoli* as chlamydospores in soil. Phytopathology 51(5): 308–312.

Outram MA, Jones EE, Jaspers MV, Ridgway HJ 2014. Development of a PCR-RFLP method to distinguish species within the *Ilyonectria macrodidyma* complex. New Zealand Plant Protection 67: 152–156.

Pathrose B 2012. Characterising sub-species variation in New Zealand *Cylindrocarpon* species that cause black foot of grapevines. Unpublished PhD thesis, Lincoln University, New Zealand.

Pathrose B, Jones EE, Jaspers MV, Ridgway HJ 2014. High genotypic and virulence diversity in *Ilyonectria liriodendri* isolates associated with black foot disease in New Zealand vineyards. Plant Pathology 63: 613–624.

Probst CM 2011. Cylindrocarpon black foot disease in grapevines: identification and epidemiology. Unpublished PhD thesis, Lincoln University, New Zealand.

Probst CM, Ridgway HJ, Jaspers MV, Jones EE 2019. Pathogenicity of *Ilyonectria liriodendri* and *Dactylonectria macrodidyma* propagules in grapevines. European Journal of Plant Pathology. 154: 405-421.

Taylor GS 1964. *Fusarium oxysporum* and *Cylindrocarpon radicicola* in relation to their association with plant roots. Transactions of the British Mycological Society 47: 381–391.

Úrbez-Torres JR, Haag P, Bowen P, O'Gorman DT 2014. Grapevine trunk disease in British Columbia: Incidence and characterization of the fungal pathogens associated with black foot disease of Grapevine. Plant Disease 98: 456–468.

©2019 New Zealand Plant Protection Society (Inc.) www.nzpps.org Refer to http://www.nzpps.org/terms_of_use.html