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Propagule and soil type affects the pathogenicity of *Ilyonectria* and *Dactylonectria* spp., the causal agents of black foot disease of grapevines

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Summary

Black foot disease of grapevines is a significant economic issue for the viticulture industry worldwide. The disease is mainly associated with soil borne pathogen species within the genera *Dactylonectria* and *Ilyonectria*. The aim of this study was to determine the pathogenicity of different pathogen propagules, including chlamydospores, conidia and mycelium, to grapevine rootstocks grown in soil. A combination of nine isolates belonging to *Dactylonectria* and *Ilyonectria* genera, representative of the fungal species associated with black foot disease in New Zealand were used to inoculate grapevines in a field experiment. In the second experiment, the pathogenicity of the different propagules was assessed in different soil types, clay loam, silt loam and sandy loam soils. In the field experiment, chlamydospores and conidia resulted in higher disease incidence and severity at 0 cm above the grapevine stem base compared with mycelium. At 5 cm above the stem base, chlamydospores caused the greatest disease incidence compared with the other two propagules. Propagule type had no effect on shoot and root dry weights. In the pot experiment, soil type affected disease incidence and severity, with clay loam soil resulting in significantly greater disease incidence and severity than silt loam or sandy loam soils. Disease severity at 0 cm above the stem base was significantly higher with conidial inoculations compared with chlamydospore inoculations irrespective of soil type. Root dry weights were also affected with heavier roots from plants grown in sandy loam compared with silt loam and clay loam soils, however, shoot dry weight was greater in clay loam and sandy loam compared with silt loam soils. The results of the study confirmed that all propagule types were able to infect grapevine rootstocks when planted in inoculated soil and showed that although the pathogens were capable of infecting the rootstocks in all soil types, disease level was higher in the heavier clay loam soil. It is therefore recommended that growers either avoid planting in such soils or apply strategies to improve drainage and soil aeration.

Key words: '*Cylindrocarpon*'; *Dactylonectria macrodidyma*; *Ilyonectria europaea; Ilyonectria liriodendri*; *Ilyonectria pseudodestructans*; disease incidence; disease severity; soil-borne pathogens.

Introduction

Black foot disease of vines is of worldwide importance, causing decline and death of young vines in the nursery or within the first few years following planting in vineyards (Halleen *et al*. 2006). The resulting loss of productive vines and the costs associated with replanting is a major economic issue for the viticulture industry. Infected vines often have reduced vigour, shortened internodes and sparse chlorotic foliage. Below ground, sunken necrotic lesions are observed on the roots and dark purple to reddish brown streaks in the wood tissue which start at the base of the rootstock and spread upward and often cause the death of the vine. When vines less than 10 years of age are infected with black foot pathogens, Scheck *et al.* (1998) concluded that death seemed to be inevitable. The disease is caused by soil borne pathogens belonging to a number of different fungal genera including '*Cylindrocarpon*', *Cylindrocladiella*, *Ilyonectria* and *Dactylonectria* species (Cabral *et al*. 2012a and b, Jones *et al*. 2012, Agustí-Brisach and Armengol 2013, Lombard *et al*. 2014, Úrbez-Torres *et al*. 2014). In New Zealand, a survey of declining vines identified *I. liriodendri*, several species within the *I. radicicola* species complex (namely *I. europaea* and *I. pseudodestructans*) and *D. macrodidyma* species complex, with *D. macrodidyma sensu stricto* (*s.s.*) being the dominant species in the latter (Pathrose 2012 and 2014, Outram *et al*. 2014).

The pathogens associated with black foot have a similar disease cycle, infecting grapevine roots and stem bases from soil borne inoculum. The pathogens can also infect grapevine propagation material through wounds, such as those due to the incomplete callusing of the stem base or wounded roots. Probst *et al.* (2019a) reported that although both rooted (where the roots were wounded) and callused grapevine rootstocks planted in inoculated potting mix resulted in infection,

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disease incidence and severity was higher for the callused rootstock material. Propagules produced on infected roots and trunk bases either spread to infect neighbouring plants by movement of soil, water or machinery or remain in soil as inoculum after removal of an infected crop (Bonfiglioli 2005, Cardoso *et al*. 2013, Berlanas *et al.* 2017). However, the biology and etiology of these soil borne pathogens in relation to grapevine infections are still poorly understood. Most *Dactylonectria* and *Ilyonectria* species associated with black foot disease are reported to produce three different type of propagules, *i.e.* mycelium within crop debris, conidia (macro- and micro-conidia) and chlamydospores which act as primary and secondary inocula (Agustí-Brisach and Armengol 2013). Probst *et al.* (2019a) reported that for *I. liriodendri* and *D. macrodidyma* all three propagule types caused infection of grapevine rootstocks. However, this study and others (Rego *et al*. 2001, Alaniz *et al.* 2010, Cabral *et al*. 2012c, Pathrose *et al*. 2014, Úrbez-Torres *et al*. 2014) investigating the pathogenicity of fungi associated with black foot disease have generally used vines grown in autoclaved soil or potting mixture, and vines and/or potting media inoculated with a conidium suspension to prove Koch's postulates. With this background, the experiments were carried out in potting mix or sterilised soil, they do not allow for the natural interactions between the pathogens and other microbes present in soil and may not reflect their true pathogenicity in soil (Abawi and Lorbeer 1972, Moreno-Velandia *et al*. 2019).

Black foot pathogens have been isolated from roots and rootstock trunks of grapevines in nurseries and in vineyards (Halleen *et al.* 2003, Gimenez-Jaime *et al.* 2006, Dubrovsky and Fabritius 2007, Cardoso *et al*. 2013, Carlucci *et al.* 2017) but the associations between soil types and symptom development have not been investigated. Berlanas *et al*. (2017) reported a positive relationship between calcium carbonate levels and the population of black foot pathogens, as colony forming unit counts, in soil. However, the authors did not investigate whether this resulted in any difference in disease levels in grapevines planted in these soils. General observations have also indicated that heavy and waterlogged compacted soils seem to cause greater grapevine losses due to '*Cylindrocarpon*' spp. (Maluta and Larignon 1991, Gubler *et al*. 2004, Halleen *et al*. 2007). This, however, is mostly based on anecdotal observations as there have been no studies comparing the effect of soil type on disease incidence caused by different black foot propagules. Therefore, the objectives of this study were to determine the pathogenicity of different *Dactylonectria* and *Ilyonectria* propagules in soil by inoculating grapevine rootstocks grown in a field site, and to determine the pathogenicity of the different propagules in different soil types.

Material and Methods

Fungal isolates and inoculum production: Nine isolates were obtained from the Lincoln University Plant Pathology culture collection and originally isolated from black foot symptomatic grapevine roots and trunks from the different New Zealand grape growing regions (Bleach *et al.* 2006). The isolates have been identified by molecular methods (Pathrose 2012, Pathrose *et al.* 2014, Outram *et al.* 2014) and deposited in the International Collection of Microorganisms from Plants (ICMP) at Manaaki Whenua – Landcare Research. *Dactylonectria macrodidyma s.s.* isolates ICMP 16788, ICMP 16789 and ICMP 16791, *I. liriodendri* isolates ICMP 16795, ICMP 16790 and ICMP 16793, *I. europaea* isolates ICMP 16794 and ICMP 16787, and *I. pseudodestructans* isolate ICMP 16792 were used to produce mixed isolate inoculum for this study. The isolates were stored as mycelial discs in glycerol at -80 °C and subcultured onto potato dextrose agar (PDA; Oxoid Ltd, Basingstoke, Hampshire, England) plates and incubated at 20 °C for 2-4 weeks.

Conidial and chlamydospore suspensions (10^6 spores·mL^{-1}) were prepared as described in Probst *et al.* (2019a). To obtain conidial suspensions, the isolates were grown on PDA at 20 °C for 3 weeks in the dark. The conidia were suspended by adding 5 mL of sterile water with 3 drops·L^{-1} of Tween 80 (polyoxylethylene (20) sorbitan mono-oleate; BDH Chemicals Ltd, Poole, England) to the plate surface, scraping the culture surface with a sterile glass microscope slide and sieving the solution through a sterile 150 µm mesh sieve. For each isolate, chlamydospores were produced in 1 L flasks containing 500 mL of 1/3 strength Czapek's dox broth (CDB; Sigma Chemicals, St. Louis, USA) inoculated with five mycelium plugs (5.5 mm diameter) cut from the growing edge of a PDA colony. The inoculated flasks were incubated on a shaker at 100 rev·min^{-1} at room temperature for 30 d, during which time mycelium initially grew in the broth and later produced chlamydospores. The chlamydospores were harvested by homogenising the mycelium for 2 min in a blender (Sunbeam Multiblender) at high speed and sieving the resulting homogenate to remove the mycelium. For each isolate the spore concentrations in the resulting conidial and chlamydospore suspensions were adjusted to 10^6 spores·mL^{-1} based on haemocytometer counts and a mixed isolate conidial or chlamydospore inoculum was prepared by mixing together equal volumes of each isolate conidial or chlamydospore suspension.

For the mycelial inoculum, the same isolates were grown on autoclaved wheat grains inoculated with five mycelial discs taken from the actively growing margins of each isolate (one isolate/inoculated flask). Flasks were incubated at 20 °C in the dark for 14 d, at which time they were visually assessed as being well-colonised by mycelium but with very few to no conidia formed. During the incubation period, the flasks were shaken daily by hand (3-5 s) to facilitate colonisation. Infested wheat grain from each isolate was mixed together in equal quantities as described in Probst *et al.* (2019a). For the controls, autoclaved water or autoclaved wheat grains were used.

Pathogenicity of propagules in field conditions: Grapevine cuttings of the rootstock variety 101-14 were obtained from a commercial nursery and were callused by placing in 20 cm deep vermiculite in a 27 °C growth chamber for 4 weeks and then hardened off in a greenhouse (14-28 °C) for 2 weeks. The planting site, selected at the Horticulture Research Area of Lincoln University, had a soil type (Templeton silt loam) that was classified as

a mottled immature pallic soil. Prior to planting, the 2.9 × 12.9 m plot of land was ploughed, rotary hoed and three planting rows were prepared, separated from each other by 0.6 m. Each planting row constituted two blocks with a 0.5 m inter-block spacing, laid out in a randomised design. Each of the treatment plots comprised 20 vines planted in a double row with a 0.1 m inter-vine spacing. The different treatment plots were separated by 0.3 m spaces arranged randomly in each block, resulting in 6 replicate plots of each treatment. The five treatments set up included vines inoculated with i) mixed isolate conidial suspension, ii) mixed isolate chlamydospore suspension, iii) mixed isolate colonised wheat grains, iv) water control, and v) autoclaved uncolonised wheat grain control.

At planting, 7 cm deep planting holes were infested with either 20 mL of conidium or chlamydospore suspensions (10^6 spores·mL^{-1}) which were spray injected with a veterinary animal dosing gun (NJ Phillips Pty Ltd, NSW, Australia) or by adding 5 g of infested wheat grains into the hole. The controls (water or autoclaved wheat grains) were treated similarly. The plants were immediately placed into the holes and left to grow for 6 months. Weeds were regularly hand removed until assessment and plants were drip irrigated as indicated by the dryness of the soil during the summer period. The temperature fluctuated between -1.4 and 34.6 °C during that period, with an average of 15.8 °C.

Assessments: After 6 months growth, the plants were uprooted one plot at a time and thoroughly washed with running tap water. The roots and shoots were removed and air-dried separately at 60 °C to constant weight. The lower stem sections, cut to a length of 20 cm were surface sterilized as described by Probst *et al.* (2019a) in batches of the same treatment and air dried for 10 min in a laminar flow cabinet. Before pathogen isolation, the root crown comprising the lowest approximately 1 cm of the stem base was discarded. An approximately 2 mm transverse piece of tissue was sliced from the basal end of the stem (0 cm) and cut into four pieces of approximately 3 mm^2, which were placed on a plate containing PDA with chloramphenicol. An approximately 2 mm transverse piece of stem was also sliced at 5 cm above the base, cut into two pieces and one piece was placed in the centre of the same plate. The plates were incubated for 7 d at 20 °C in the dark and the presence of *Dactylonectria* and/or *Ilyonectria* species growing from the wood samples at 0 and 5 cm above stem bases was determined. The presence of the pathogens in the trunks at 0 cm or 5 cm was taken as evidence of disease incidence and the proportion of wood pieces (out of four) at 0 cm colonised by the pathogens as disease severity (Probst *et al.* 2012).

Pathogenicity of propagules in different soil types: Grapevine cuttings of the rootstock variety 101-14 obtained from a commercial nursery were callused for a month as previously described. The three different soil types selected from Lincoln University's surroundings were classified as heavy (Wakanui clay loam), medium (Templeton silt loam) and light (Eyre shallow fine sandy loam). The pH of the soils were all approximately 6.0. The soils were transported from the different sites to Lincoln University's nursery area in 200 L containers and used the next day. Large stones and plant debris were removed and any large clumps of soil were broken up. Conidial and chlamydospore suspensions, infested wheat grains and their respective controls were prepared as described for the previous experiment. The mixed isolate conidial, chlamydospore suspensions or infested wheat grain inocula of the nine isolates were used as inocula as described for the previous experiment.

Individual pots (2.5 L) were filled with one of the three soil types, and 7 cm deep holes made in the centre which were inoculated with 20 mL of spore suspension or 5 g of colonised wheat grains as described for the previous experiment. The controls were inoculated with the equivalent amount of sterile water or autoclaved wheat grain. The callused plants were then planted into the holes and the 20 replicate vines per treatment were laid out on mesh tables in a greenhouse in a split-plot design. High pressure sodium lamp (Son-T Agro 400, Philips) lights were turned on from 4 am to 12 pm and from 4 pm to 8 pm for the duration of the experiment to ensure plants were under a 16 h light exposure. Temperatures varied between 14 °C and 30 °C during the length of the experiment. The pots were weeded during the duration of the experiment, and the plants lightly watered daily. After 6 months growth the vines were assessed as previously described.

Statistical analysis: The disease incidence (0 and 5 cm above the stem base) data were analysed by logistic regression for binomial data (presence or absence of pathogen) to test the significance of the different factors (propagule type, soil type or interaction) followed by pairwise comparisons between individual treatments within the tested factors to determine the significance of their differences using Fisher's exact test. The analysis of disease severities (0 cm above the stem base) and root and shoot dry weights was completed with a general linear model with terms appropriate to the design, to determine effects of the individual factors of interest and the two-way interactions amongst them. Where significant main effects or two-way interactions were identified, the significance of difference between related treatments was further explored using Fisher's protected Least Significant Difference tests. A P-value of ≤ 0.05 was used to indicate statistical significance. All data were analysed using Statistical Package for Social Sciences (SPSS) version 13.0.

Results

Pathogenicity of propagules in field conditions: There was a significant effect ($P < 0.001$) of propagule type on disease incidence at 0 cm above the stem base. There was no significant difference in the disease incidence caused by chlamydospores and conidia, with both causing significantly greater disease incidences than mycelium, with means of 84.4%, 82.3% and 65.5%, respectively (Fig. 1). All propagules caused mean disease incidences significantly greater than their respective controls (water control, 40.0 % and wheat control, 20.5 %).

There was a significant effect ($P < 0.001$) of propagule type on disease incidences at 5 cm above the stem base. Inoculation with chlamydospores caused significantly greater

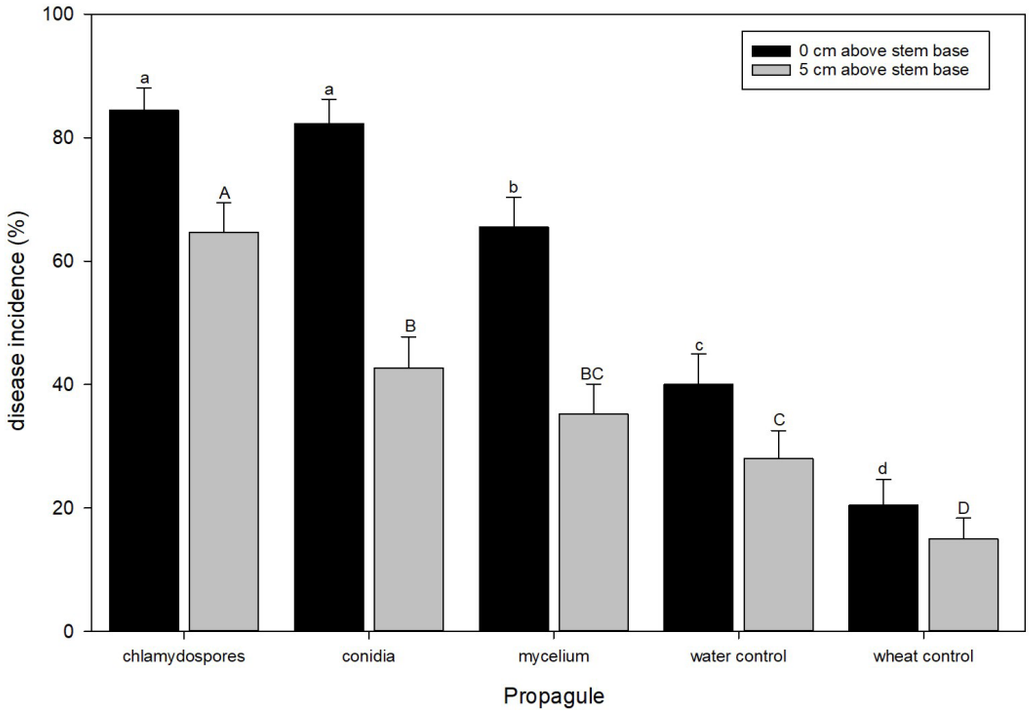

Fig 1: Mean disease incidence at 0 and 5 cm above stem bases of 101-14 grapevine rootstock plants, 6 months after inoculation with propagules from *Ilyonectria/Dactylonectria* spp. in the field experiment. Means of 6 replicate plots each containing 20 vines per plot. Bars with different letters (a-d for disease incidence at 0 cm and A-D for disease incidence at 5 cm) indicate values which are significantly different ($P \leq 0.05$) from one another. Error bars represent standard error.

disease incidences at 5 cm above the stem base than conidia and mycelium which did not differ significantly from each other, with means of 64.6 %, 42.7 % and 35.2 %, respectively. All propagules caused significantly greater disease incidences than their respective controls (water control, 28 % and wheat control 15.0 %) (Fig. 1).

Disease severities at 0 cm above the stem base differed significantly ($P<0.001$) between propagule types. Chlamydospores (70.8 %) and conidia (69.3 %) caused similar disease severities which were significantly greater than for mycelium (52.4 %). Disease severities of plants inoculated with propagules were significantly greater than those of plants treated with the respective controls (water control, 26.6 % and wheat control, 13.3 %).

For the root and shoot dry weights, there were no significant differences ($P = 0.334$ and $P = 0.054$, respectively) between treatments. Mean root dry weights ranged between 2.2 g for the plants inoculated with mycelium and 3.5 g for control plants treated with water. The mean shoot dry weights ranged between 1.7 g for control plants inoculated with autoclaved wheat grains and 2.5 g for control plants inoculated with water.

Pathogenicity of propagules in different soils: The disease incidence at 0 cm in the untreated control treatments (5 % and 0 % for sandy loam, 15 % and 15 % for silt loam and 20 % and 25 % for clay soil, for the water and wheat controls, respectively) indicated a background population of black foot disease in the soils. There was no significant interaction ($P = 0.639$) between soil type and inoculation treatment on disease incidence at 0 cm above the stem base. The main effect of soil type significantly affected ($P = 0.001$) disease incidences at 0 cm above the stem. Mean disease incidences were significantly greater for clay loam (71.0 %) than for sandy loam (46.0 %) and for silt loam (51.0 %), but the last two soils did not differ significantly. Disease incidences at 0 cm above the stem base were significantly affected ($P < 0.001$) by inoculation treatment. All propagule types caused significantly greater disease incidences than their respective controls (26.7 % for water control and 16.7 % for wheat control). However, there was no significant difference in disease incidence between propagule types, with means of 73.3 % for chlamydospores, 85.0 % for conidia and 78.3 % for mycelium (Fig. 2).

There was no significant interaction ($P = 0.649$) between soil types and inoculation treatments on disease incidences at 5 cm above the stem base. There was a significant difference ($P < 0.001$) in the disease incidences at 5 cm above the stem base between soil types. The mean disease incidence at 5 cm was significantly greater for clay loam (54.0 %) than for sandy loam (30.0 %) and for silt loam (37.0 %). Disease incidences at 5 cm were significantly different ($P < 0.001$) between propagule types. Conidia caused greater disease incidences at 5 cm than chlamydospores and mycelium, with means of 68.3 %, 53.3 % and 50.0 %, respectively (Fig. 2). All propagule types caused disease incidences significantly greater than their respective controls (18.0 % for water control and 11.7 % for wheat control).

There was a significant difference ($P<0.001$) in the mean disease severities at 0 cm above the stem base between soils, being significantly greater for clay loam (61.0 %) than for silt loam (38.7 %), with both being significantly greater than sandy loam (31.0 %). Disease severities at 0 cm above stem bases showed significant differences ($P < 0.001$) between propagule types. Conidia caused greater disease severity

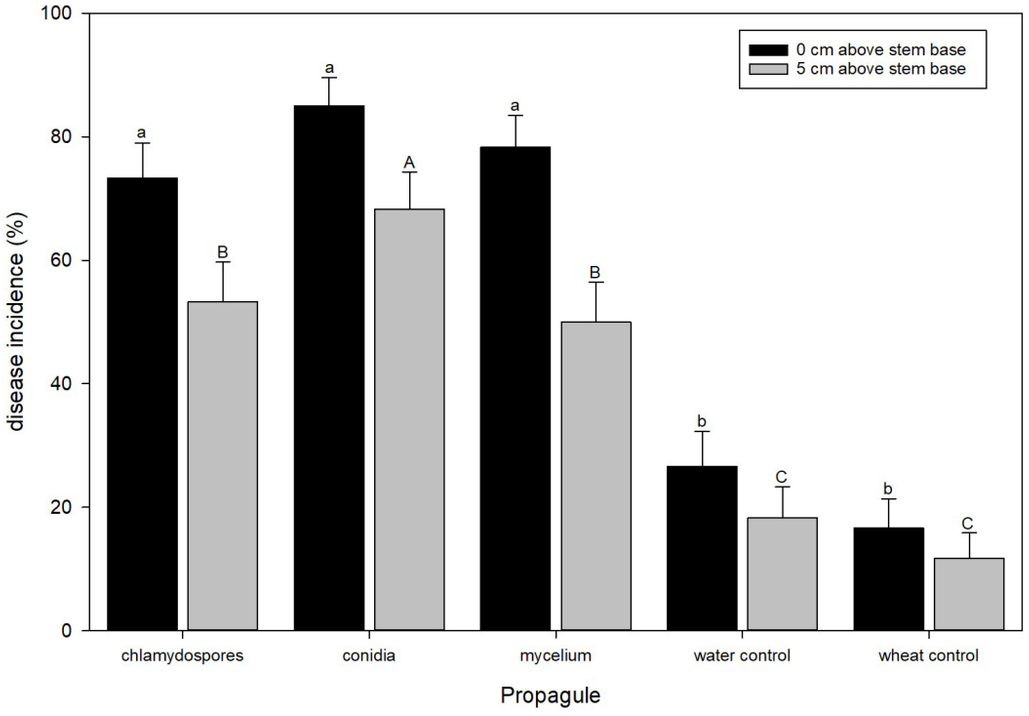

Fig 2: Disease incidence at 0 and 5 cm above the stem bases of 101-14 grapevine rootstock plants, 6 months after inoculation with three propagule types from *Ilyonectria/Dactylonectria* spp. and grown in different soils in a pot experiment. Bars with different letters (a-b for disease incidence at 0 cm and A-C for disease incidence at 5 cm) indicate values are significantly different from one another ($P \leq 0.05$) across the different soil types. Error bars represent standard error.

than chlamydospores, however, mycelium caused a similar disease severity to the two other propagules, with means of 72.5, 60.8 and 55.4 %, respectively. All propagules caused significantly greater disease severities than their respective controls (water control 17.5 % and wheat control 11.7 %).

For the root dry weights, there were significant effects of soil types ($P < 0.001$), of the interaction between propagule types and soil types ($P < 0.001$) and of propagule types (P=0.045). For the different soil types, the mean root dry weight was greater for plants grown in sandy loam (14.5 g) followed by those grown in clay loam (10.9 g) which were greater than for silt loam (7.4 g) (Fig. 3A). The interaction between soil and propagule types was associated with the similar root dry weights for mycelium inoculations for all soil types but root dry weights differed for other propagules and the controls. The root dry weights ranged from 10.6 g for mycelium to 11.6 g for chlamydospores and were not significantly different between propagules and their respective controls, however, grapevines inoculated with water had significantly greater mean root dry weight (11.2 g) than plants inoculated with autoclaved wheat grains (9.6 g).

For the shoot dry weights, there were significant differences between soil types ($P < 0.001$) and a significant interaction between soil types and propagule treatments ($P = 0.009$). The shoot dry weights varied between 0.9 and 1.2 g for plants grown in silt loam, between 2.0 and 2.9 g for those grown in clay loam and between 1.6 and 2.7 g for those grown in sandy loam (Fig. 3B). For the different soil types, the mean shoot dry weights were significantly lower for grapevines grown in silt loam (1.0 g) than those grown in sandy loam (2.1 g) and clay loam (2.5 g). The interaction between soil and propagule types was associated with the similar effect of mycelium in sandy and silt loams, which differed from other propagules.

Discussion

This is the first study to investigate the relative capacity of the different propagules produced by *Dactylonectria* and *Ilyonectria* species associated with black foot disease to infect grapevine rootstocks planted in soil, and showed that all of the propagules were infectious. The study confirmed previous results whereby inoculation of grapevine rootstocks grown in potting mixture with chlamydospores, conidia or mycelium of both *D. macrodidyma* and *I. liriodendri* resulted in infection, with conidial inoculation causing greater disease incidence (Probst *et al.* 2019a).

Under field conditions chlamydospores and conidia were shown to cause significantly higher disease incidences at 0 cm above the stem base than mycelium (Fig. 1), whereas in the pot experiment with the different soil types disease incidence at the stem base was similar for the different propagule type (Fig. 2). This indicated that the mycelium added to the planting holes in infested wheat grain were less infective under field soil conditions. Fragments of mycelium used in this inoculation method could be more subject to lysis, under field conditions, compared with conidia and chlamydospores (Taylor 1964, Probst *et al.* 2019b). It was also shown by Probst *et al.* (2019b) that in the absence of a host plant mycelial inoculum buried in soil rapidly converted into conidia, which then subsequently convert into survival chlamydospores. It remains unclear if mycelial inoculum directly infects plants under soil conditions or whether the

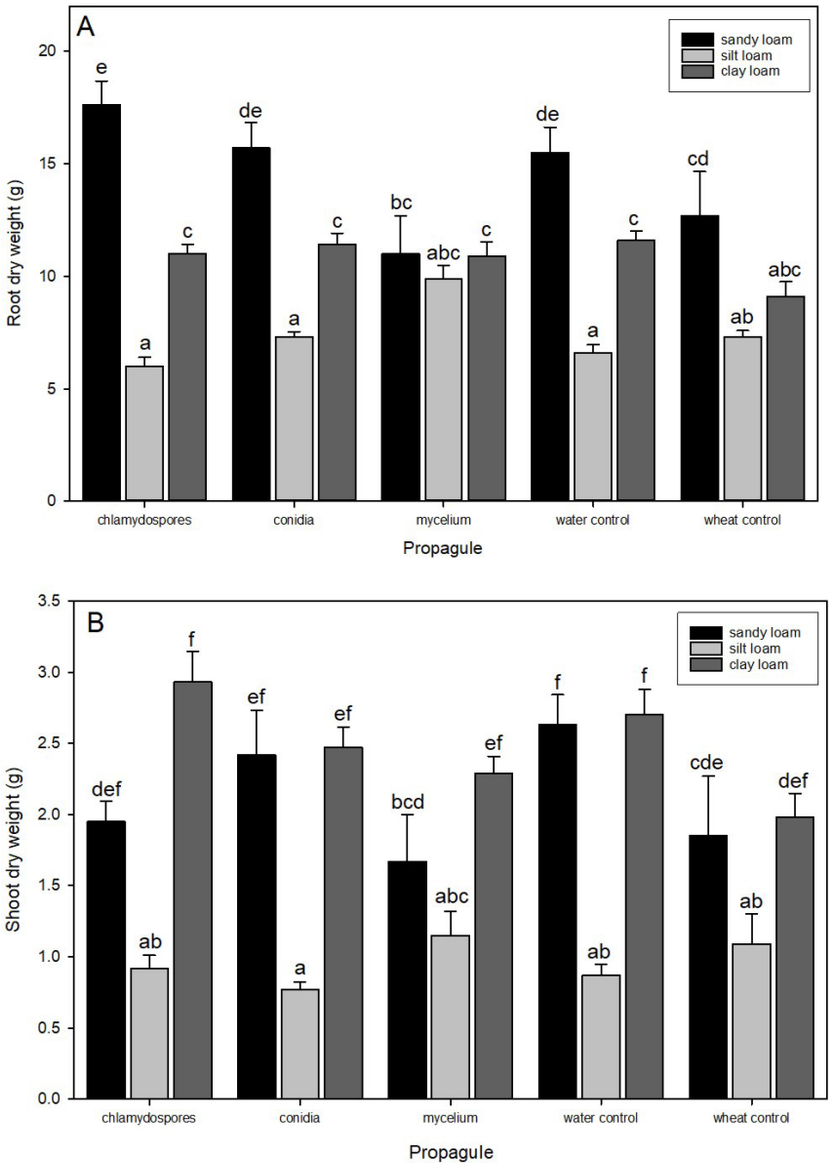

Fig 3: Mean (**A**) root and (**B**) shoot dry weights of 101-14 grapevine rootstock plants, 6 months after inoculation with three propagules from *Ilyonectria/Dactylonectria* spp. in three soil types. Bars with different letters indicate values are significantly different ($P \leq 0.05$) from one another. Error bars represent standard error.

same process occurs in the presence of a host by converting into conidia and/or chlamydospore in response to root exudates. If the latter is the case then this is likely to delay the infection of the host compared with conidial inoculum which would be able to respond rapidly to infect the host. Further, conversion of mycelium to conidia/chlamydospores was seen by Probst *et al.* (2019b) to result in reduction in inoculum levels, and this, along with any degradation of the mycelium in the soil environment, would also lead to reduced inoculum levels.

In the pot experiment disease severity at 0 cm, and disease incidence at 5 cm above the stem base (Fig. 2) was higher for conidium compared with both mycelium and chlamydospore inocula, indicating that conidium inoculum resulted in faster infection of the rootstocks and therefore colonisation progressed further up the stem base. However, in the field experiment disease incidence at 5 cm was higher for chlamydospore inocula compared with conidia and mycelium (Fig. 1), indicating in this case infection was quicker for chlamydospore inocula. The reason for this difference is unclear, but maybe related to difference in the response of the spore types to environmental conditions. Conidia, considered primarily as dispersal spores in the disease cycle of black foot, in comparison to survival related chlamydospores (Agustí-Brisach and Armengol 2013), may be able to more rapidly respond to plant signals including root exudates, resulting in rapid infection. However in the presence of sub optimum conditions, as potentially seen in the field, chlamydospores may be able to more rapidly respond. The effect of the soil environment on pathogenicity is very complex, with many soil factors including proximity to the host, and nutrient and physical environment as well as interactions with microbial communities likely to influence the relative infectivity of the different propagule inocula (Raaijmakers *et al*. 2009). Although the present study showed that all propagules are able to infect grapevines

as soil inocula, further studies are required to determine the relative risk of different soil borne inocula on disease incidence in nursery and vineyard soils.

Disease incidence was relatively high (65-85 %) in this study, and was similar to that observed in an experiment with inoculated plants grown in potting mix (Probst *et al.* 2019a). In both studies the inoculum was placed under the plants and therefore in close contact with the plant for infection. However, under more natural conditions where the inoculum would be distributed in the soil rather than directly placed under the plant would likely result in lower disease levels compare with what was seen in the current study.

The relative pathogenicity of the different propagules in the three soil types was not influenced by the different soil types as there was no interaction observed between propagule treatments and soil types. However, soil type was seen to affect disease levels, with higher disease incidences and severity in the heavy clay loam soil compared with the medium silt loam and light sandy loam soils. Clay soils retain more water, dry out and warm up slowly whereas sandy soils dry out and warm up more quickly (Bruehl 1987). Black foot disease has been observed to develop preferentially in poorly drained heavy soils and particularly in areas where plants were waterlogged (Maluta and Larignon 1991, Gubler *et al.* 2004, Bonfiglioli 2005, Halleen *et al*. 2007) and this study provides evidence to support these field observations.

A background level of *Dactylonectria/Ilyonectria* was observed infecting the uninoculated control treatments in the field experiment and in the silt loam soil in the pot experiment which originated from the same location. It was responsible for 40.0 and 15.0 % disease incidences at 0 cm above stem bases for the plants inoculated with sterile water and grown in the field and silt loam treatment in the pot experiment, respectively. The site was previously used as an apple orchard and supports the hypothesis that *Dactylonectria/Ilyonectria* propagules can accumulate in orchards in New Zealand and can infect subsequently planted grapevine plants (Bonfiglioli 2005). *Dactylonectria* and *Ilyonectria* spp. associated with black foot disease are reported to be linked to tree decline in apple orchards (Tewoldemedhin *et al*. 2011, Manicni *et al*. 2018) indicating previous cropping history needs to be taken into account when planting new vineyards or nursery sites to minimise the risk of black foot disease. Although the other two soils used in the study did not have a history of grapevine or horticultural fruit crops, being sampled from sites under long-term cropping and pasture, background infection by black foot pathogens was still observed. The fungal species associated with black foot disease are pathogens of a wide range of hosts and are also saprophytes able to utilise a wide range of substrates, and as such are commonly found in soil (Berlanas *et al*. 2017). As well as being associated with root and stem rots of horticultural fruit crops such as apple (*Malus domestica;* Manici *et al.* 2018), avocado (*Persea americana;* Vitale *et al*. 2012), kiwifruit (*Actinidia chinensis*; Erper *et al*. 2013) they are reported as pathogens of pasture species such as clover (*Trifolium* spp.) and lucerne (*Medicago sativa*) and other crops including peas (*Pisum sativum*) and beans (*Vicia faba* or *Phaseolus* spp.) (Lager and Gerhardson 2002), and have recently been identified as root pathogens of beet (*Beta vulgaris*; Chand, Jones and Casonato, unpubl.). Weeds are also asymptomatic hosts of black foot pathogen species (Agustí-Brisach *et al.* 2011). Rego *et al.* (2009) reported the proportion of grapevine plants infected by '*Cylindrocarpon*' spp. was high in a nursery field where grapevines had been planted for 2 years, followed by 3 years of rotation with other crops including potato, cabbage, carrot, garlic, leek and cereals. Further, Cardoso *et al.* (2013) and Berlanas *et al.* (2017) detected black foot pathogens in grapevine nursery soils during the rotation period with wheat and barley. Similarly, Bleach (2013) reported higher infection of rootstocks planted in black foot pathogen infested soil after a wheat green manure crop compared with the untreated bare fallow treatment, where the land remained uncropped for a season and kept free from vegetation by cultivation. This supports the suggestions by Halleen *et al.* (2003) that cover crops included in standard grapevine nursery rotation systems may result in a build-up of black foot pathogen inoculum. It is likely therefore that many crops as well as weeds and other organic material in these field sites support the population of these pathogens.

Plant growth, as determined by both root and shoot dry weights, was not affected by propagule treatments but was affected by soil type. This is likely to be a result of physicochemical differences in the soils. For example, root weight was higher in the light sandy loam soil than in heavy clay loam and medium silt loam soils with Tomasi *et al.* (2007) reporting that root development in grapevines was greater in sandy soil where the roots needed to search more deeply for water. This principle would indicate lowest root weights for heavy soil which was not seen in the current study, however the soils were well aerated at the beginning of the experiment, which may have allowed greater drainage and accounted for the extensive root systems after 6 months. Plants grown in the field site (medium soil) had the lowest root dry weights, which was probably due to the less optimum growth conditions in the field compared with the greenhouse, including greater temperature fluctuation experienced by the vines.

The present study has provided information which improves our understanding of the etiology and pathogenicity of black foot pathogens in soil. All propagule types could infect grapevines with disease incidence greater with conidia and chlamydospores compared with mycelium. The study also showed that heavy soils facilitate *Ilyonectria/ Dactylonectria* infection compared with lighter soil types, indicating grapevines planted on heavy soils are at higher risk of developing black foot disease compared to plants grown on light soils. Clay soils retain more water than sandy soils which can lead to waterlogging and reduced aeration in the root zone, causing plant stress whilst favouring root pathogens (Cook and Papendick 1970). Further, heavy soils are more likely to contract when water is unavailable and damage roots providing an entry site for these wound pathogens. However, as the pathogens were shown to be capable of infecting in all soil types, growth of vines on a particular soil type does not eliminate the threat, although light soils are likely to reduce its occurrence. Gubler and Petit (2013) suggested that in heavy soils vines should

be planted on raised beds to increase drainage, with drip irrigation emitters placed away from the vine base. Further, strategies to improve the structure of heavy soil through addition of compost or mulches to improve water drainage and soil aeration should be investigated to reduce the impact of black foot disease in nurseries and when new vines are out planted in the vineyard.

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