Three "other" diseases impacting avocado productivity in Australia

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INTRODUCTION

Phytophthora root rot is undoubtedly the single most important constraint to orchard productivity in Australia (and probably South Africa too). However, in recent years the industry in Australia has also faced significant tree deaths and/or yield reductions resulting from other fungal pathogens, viz. black root rot, brown root rot and branch dieback. The purpose of this article is to heighten awareness in the South African industry to these disease problems.

Black root rot is a severe disease of young avocado trees caused by soilborne fungal pathogens in the Nectriaceae family (Dann *et al.,* 2012; Ramírez Gil, 2013; Vitale *et al*., 2012). The most severe symptoms of black root rot include leaf wilt and chlorosis and rapid decline and death of young orchard transplants. Affected roots have distinct brown to black, sunken lesions. There have been several reports of fungi associated with avocado black root rot, however, there is considerable confusion with respect to taxonomy and nomenclature (names) of causal agents. *Ilyonectria destructans* associated with avocado was first reported by Joe Darvas (1978) and subsequently others as *Cylindrocarpon destructans* (Besoain and Piontelli, 1999; Ramírez Gil, 2013) and as *Neonectria radicicola* (Zilberstein *et al.*, 2007). Species confirmed by pathogenicity tests as the cause of black root rot in avocado include *Calonectria ilicicola* in Australia (Dann *et al.,* 2012), which also caused severe stunting, and *Dactylonectria macrodidyma* (as *Ilyonectria macrodidyma*) in Italy (Vitale *et al.*, 2012). *Ilyonectria liriodendri*, and an undescribed *Gliocladiopsis* sp. were not pathogenic to avocado seedlings in glasshouse pathogenicity tests (Dann *et al.*, 2012). A clear understanding of the taxonomy and pathogenicity of these fungi is necessary for accurate diagnosis and management strategies.

Brown root rot is caused by the basidiomycete fungus *Phellinus noxius*, and is characterised by rapid leaf wilting and tree death, with a fungal crust or "stocking" sometimes evident on the trunk (Dann *et al.,* 2013). This fungus has an extremely wide host range amongst woody species in tropical and subtropical environments, and has been reported in many African countries including Cameroon, Kenya and the Democratic Republic of the Congo, but not, to our knowledge, in South Africa. In avocado, slow root-to-root spread results in successive death of trees along the row. The disease is most effectively managed by removal of the dead tree and its immediate neighbours, and installation of root barriers. Macro-injection with fungicides is problematic and not commercially feasible for avocado orchards. Our recent research efforts have evaluated susceptibility of macadamia, mango, passionfruit and citrus to *P. noxius*, and tested *Trichoderma* spp. as potential biocontrol agents, and is described below.

Beetle-vectored branch dieback was first observed in southern Queensland in 2010. The disease vector was identified as a small (1-2 mm) ambrosia beetle (a type of borer), *Euwallacea fornicatus.* This insect can carry two fungi, *Fusarium* sp. and *Bionectria* sp., which are pathogenic to avocado seedlings (Geering and Campbell, 2013). This beetle-fungus symbiosis is causing significant damage to both declining and healthy trees in north Queensland (Geering and Campbell, 2013), California and Israel (Freeman *et al.,* 2013). In California, more than one beetle and fungal species are associated with die-back symptoms (Eskalen *et al.,* 2013; http://ucanr.edu/sites/pshb/files/238252.pdf) and further research on epidemiology, causal organisms and management options is necessary in Australia. Current management involves pruning and removing affected branches from the orchard. This beetle-disease complex will not be discussed further in this paper, and the reader is referred to the relevant publications for more information.

RESULTS AND DISCUSSION

Black root rot

A recent PhD study in Australia examined more than 150 isolates of fungi from the family Nectriaceae, which were collected from symptomatic avocado roots from nurseries and orchards, and from other host species. Bayesian inference and Maximum Likelihood phylogenetic analyses of concatenated ITS, β-tubulin and histone H3 gene loci were used to classify the isolates into six genera, viz. *Calonectria*, *Dactylonectria*, *Ilyonectria*, *Mariannaea*, *Cylindrocladiella* and *Gliocladiopsis*. Three new species of *Gliocladiopsis* have been described, *G. peggii*, *G. whileyi* and

G. forsbergii (Parkinson *et al.,* 2017). Over 20 putative novel cryptic species in these genera were discovered and remain to be formally described. *Ilyonectria destructans* was not associated with Australian avocado trees.

Glasshouse pathogenicity tests on avocado cv. Reed seedlings demonstrated *Calonectria ilicicola* isolated from avocado, papaya, peanut and custard apple were severely pathogenic to avocado, causing wilting and stunting, or seedling death, within 5 weeks after inoculation (Fig. 1). *Calonectria* sp. isolated from blueberry caused significant root necrosis but not stunting. *Dactylonectria macrodidyma*, *D. novozelandica*, *D. pauciseptata* and undescribed *Dactylonectria* sp. were also pathogenic to avocado, causing severe root necrosis but not stunting. Despite the relatively high frequency of isolation from symptomatic avocado roots, the tested *Ilyonectria*, *Gliocladiopsis* and *Cylindrocladiella* spp. isolates from avocado, and *Ilyonectria* sp. from grapevine were not pathogenic (data not shown).

Calonectria ilicicola as a pathogen of avocado remains restricted to trees originating from one source, and there were only 4 isolates collected in the recent study. However, avocado plantings into land previously

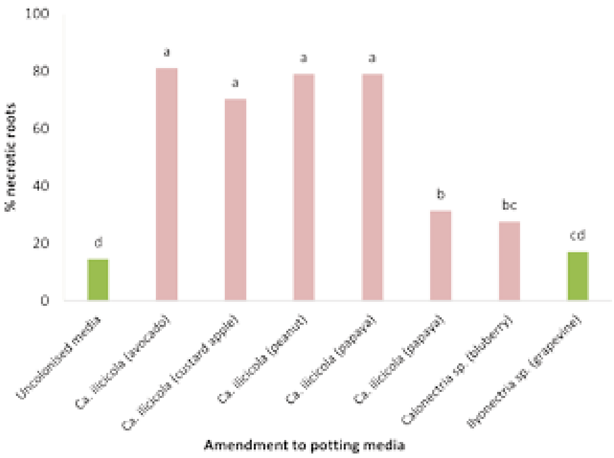

Figure 1. Effect of soil amendment with *Calonectria* and *Ilyonectria* spp. on root necrosis in avocado cv. Reed seedlings 5 weeks after inoculation.

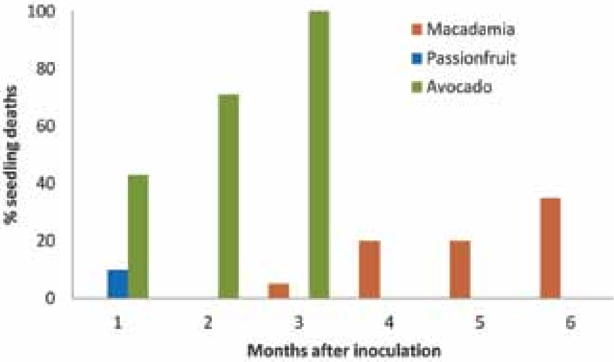

Figure 2. Mortality of avocado, passionfruit and macadamia following inoculation with *Phellinus noxius*.

cultivated to peanut are increasing. This could be a risky practice if the peanuts had Cylindrocladium black rot (CBR) disease, also caused by *Ca. ilicicola*. Conversely, *Dactylonectria* species are more commonly isolated from avocado of all ages, health types and geographical regions, and more than 50 isolates were collected. While their impact in nurseries may be obvious, further research is necessary to determine whether they are causing tree decline or reducing productivity in established orchards, either alone, or in combination with other pathogens, such as *Phytophthora cinnamomi*. *Mariannaea* spp. have not yet been tested for their pathogenicity to avocado.

Brown root rot

A glasshouse trial determined the relative susceptibility of other horticultural woody species to *Phellinus noxius*. The rationale for this was to provide information to industry about alternative horticultural crops may be planted in sites heavily infested with the brown root rot fungus. The alternate host seedlings evaluated were passionfruit (*Passiflora edulis*) rootstock #172, Kensington Pride mango, macadamia rootstock Beaumont, citrus rootstocks Troyer, Flying Dragon and Cleopatra, hoop pine (*Araucaria cunninghamii*), with Reed avocado included as a susceptible control. Ten percent of passionfruit seedlings died within one month after inoculation, but there were no further deaths. Within three months of inoculation all avocados had succumbed to *Phellinus*, and at the termination of the trial 6 months post-inoculation, almost 40% of macadamia seedlings had died (Fig. 2). No deaths occurred in mango, citrus, or hoop pine. Interestingly, despite *Phellinus* being a significant cause of tree death in commercial hoop pine plantations, inoculation with the avocado isolate used in this experiment failed to result in hoop pine seedling death, although it significantly ($P<0.05$) reduced seedling height compared to non-inoculated plants.

Surviving plants from the trial were assessed for further indications of damage to root systems and/or reductions in plant vigour. The presence of the characteristic Phellinus stocking (mycelia and soil encrusted areas on plant root systems), varied considerably among hosts. Stocking was absent on all surviving passionfruit, present in 19-35% of citrus (excluding Citrus – Flying Dragon) and mango, and 75% of surviving macadamia plants. In a small number of hoop pine and macadamia plants, tissue discolouration was evident underneath the stocking, potentially indicating early stages of pathogen growth into the plant. However, no discolouration was observed in internal tissues of mango or citrus roots underneath the stocking.

The alternate host trial data indicate that macadamia would not be a suitable alternate tree crop to replace avocado orchards affected by *Phellinus*. Although no stocking was observed on the surviving passionfruit seedlings, the deaths of two plants is consistent with a report from Thailand indicating that *Passiflora edulis* is susceptible. The presence of stockings on mango and citrus, despite the absence of plant death in the glasshouse trial, warrants further investigation under field conditions before providing recommendations to industry on replant options, particularly due to the limited numbers of confirmed tree deaths due to Phellinus in commercial orchards.

A second glasshouse experiment evaluated the viability of *Phellinus noxius* from infested root debris, after treatment with a soil fumigant, fungicide soil drenches, Brassica biofumigant, cyclical waterlogging or *Trichoderma* sp. Root debris, buried up to 1 m deep, was collected from sites in an orchard at Childers, Queensland, where trees had died from brown root rot, and been removed at least 3-4 years earlier. These roots were encrusted with the characteristic "stocking". Phellinus was successfully isolated into media from 40% of root pieces which were typically 2-4 cm in diameter, confirming the long-term survival of the pathogen in woody root debris. Infested root pieces were placed at the bottom of 4 L planter bags which were then filled with a red krasnozem soil sourced from an avocado orchard at Childers. Three and six months after initiation of the treatments root pieces were recovered and plated onto selective media and Phellinus growth recorded.

Three months after treatment, Phellinus could be recovered from root pieces subjected to every treatment except Trichoderma, although frequency of isolation was somewhat reduced for chloropicrin, biofumigation and propiconazole treatments (Fig. 3). At 6 months there was a reduction in frequency of *Phellinus* isolation from all treatments except heavy irrigation (Fig. 3), which was unexpected based on a previous study which showed that extended periods of wet soil reduced *Phellinus* viability and was a recommended management strategy (Chang, 1996). It was surprising that no *Phellinus* was recovered at 6 months from untreated and dry soil treatments. Interestingly, when the root pieces were recovered and dissected for plating onto media, there were differences in the internal colour. Root pieces recovered from untreated pots were bright with brown *Phellinus* sclerotial plates clearly evident (Fig. 4). However, root pieces recovered from chloropicrin and Trichoderma treated pots had a very dark grey internal colour. Further experiments with Trichoderma and mustard green manure crops are in progress.

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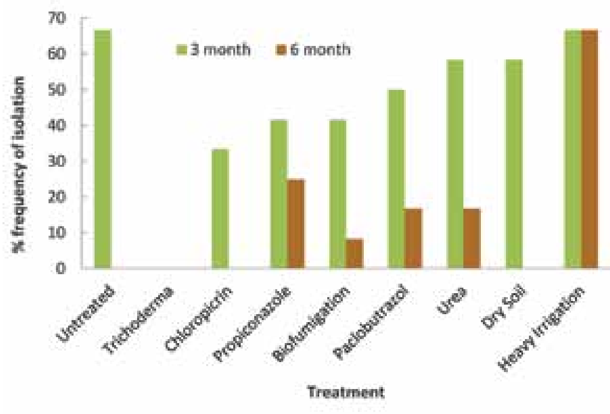

Figure 3. Frequency of isolation of *Phellinus noxius* from infested root pieces 3 and 6 months after treatment.

Figure 4. Root pieces colonised by *Phellinus noxius* buried in soil and recovered 3 months after nil treatment. The brown lines are the sclerotial plates of the fungus, evident within the spongy, decayed root tissue.

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