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FINAL REPORT 2024

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1. PROGRAMME & PROJECT LEADER INFORMATION

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2. PROJECT INFORMATION

Project title	Determining factors that may shorten the productive lifespan of grapevine rootstock mother blocks
Short title	Productive lifespan of rootstock mother plantations

Fruit kind(s)	wine grapes		
Start date (mm/yyyy)	2019-04	**End date** (mm/yyyy)	2023-03

Key words	Rootstocks, Mother vines, Productive lifespan, Fungal pathogens

	CFPA	RAISIN SA	HORTGRO	SATI	WINETECH	ARC	OTHER
TOTALS All years	R 0	R 0	R 0	R 0	R 1586681	R 1239067	R 0

Total cost of entire project	R 2825748

3. EXECUTIVE SUMMARY

Objectives and Rationale

To manage and plan for the replacement of rootstock mother blocks by determining, in parallel, the incidence of trunk disease pathogens, physiological status, and morphological dimensions of rootstock canes from mother blocks of various ages.

Methods

Conduct a survey in 41 mother blocks to determine morphological dimensions, physiological status and fungal pathogen status of one-year-old canes over three seasons.

Conduct a survey of vine heads in 38 mother blocks over two seasons for the incidence of trunk pathogens.

Determine the effect of infection on callusing and nursery vine performance.

Key Results

One-year-old canes serve as an inoculum source in newly grafted vines. Botryosphaeriaceae infection in the rootstock mother blocks results in infected 1-year-old canes. The morphological and physiological data from the one-year-old canes were influenced by season, and not by rootstock mother vine age. Significant correlations were recorded between several minerals and between minerals and cane morphology. Younger rootstock mother blocks were shown to produce more usable canes than older mother blocks. The main occurring grapevine trunk disease pathogen in rootstock mother vine heads is *Phaeomoniella chlamydospora* (Petri disease), followed by Botryosphaeriaceae spp. (Bot canker and dieback), Hymenochaetaceae spp. (Esca), Togniniaceae spp. (Petri disease), Diaporthales spp. (Phomopis dieback) and Diatrypaceae spp. (Tandpyn-like). High incidences of GTD pathogens were recorded in the heads of all rootstock mother vines, mostly irrespective of age. Vine age does not have a direct impact on the total certifiable yield or root mass of nursery vines. However, inoculum in contaminated canes originating from infected rootstock mother blocks has been shown to adversely affect nursery vine performance. The nursery process has been shown as the only source of *Pleurostoma richardsiae* inoculum in this study, which needs to be addressed.

Key Conclusion of Discussion

This study, for the first time, provided an opportunity to combine physical, physiological and GTD details on the same rootstock mother material and to provide answers that are hitherto unknown. The fungal pathogens identified from the heads and canes of mother vines are important grapevine trunk disease pathogens that can cause problems during the propagation process or in newly established vineyards. It affects the ability of the rootstock mother vines to produce healthy canes and reduces the productive lifespan of mother blocks.

Take Home Message for Industry

Grapevine trunk diseases need to be managed more effectively in rootstock mother blocks, and planning for the replacement of these blocks needs to be done at a younger age. Aerial inoculum from within rootstock mother blocks should be contained by means of sanitation. This entails the removal and destruction of any dead plants or plant material, the removal of fungal fruiting bodies, the control of insect vectors and the regular sanitation of pruning shears during the pruning process. The use of pruning wound protectors is urged to be used by the South African grapevine industry immediately after pruning of rootstock mother blocks. Whether painting or spraying, it is crucial to ensure comprehensive coverage of all wounds, including those on one-year-old wood, regardless of their size. Another strategy to combat the infection of one-year-old canes in rootstock mother blocks is to trellis rootstock mother vines.

4. PROBLEM IDENTIFICATION AND MOTIVATION

Problem Identification

"Determining the efficient lifespan of rootstock mother plantations" has been identified as a priority in the "Research strategy for SA Wine Industry – 2018", Theme 2 "Health of Vitis species", 2.1. "Grapevine Improvement", 2.1.2.3. "Phytosanitary Health".

Although the Vine Improvement Scheme makes provision for several requirements (i.e. requirements for units, phytosanitary status, physical dimensions, etc.), none of these address the productive lifespan of rootstock mother blocks. Rootstock mother vines are known as sources of grapevine pathogen inoculum, initially becoming infected through pruning wounds, where the pathogens spread to canes as spores through the xylem or by growing endophytically as mycelium into the canes, which are then used as propagation material. Currently, the risks associated with ageing rootstock mother blocks are unknown, as most of these infected rootstock canes will visually appear healthy. The aim of the study will therefore be to identify factors that may reduce/shorten the productive lifespan of rootstock mother blocks in order to provide local grapevine nurseries, rootstock mother block owners and the Plant Improvement Scheme with guidelines on firstly, how to manage rootstock mother blocks in order to minimize the risks associated with propagation material sourced from aging mother vines and secondly, to properly plan for replacement of such mother blocks in order to ensure sustainability at farm level.

Motivation

This study, for the first time, will provide physical, physiological and pathogenic detail on the same mother material in order to determine the quality of rootstock propagation material, but will also provide information on the factors that may reduce the productive lifespan of rootstock mother vines from which these shoots are harvested.

5. ACCUMULATED PROGRESS TABLE

Objectives	**Milestones (Significant event or stage in a project)**	**Date Achieved**
Survey of rootstock canes in mother blocks for the incidence of trunk pathogens	Obtain a list of certified rootstock mother blocks from the VIA and identify blocks to be surveyed. Obtain rootstock canes from mother blocks (Season 1) Conduct fungal isolation from canes Identification of fungal pathogens (morphology). Identification of fungal pathogens (molecular). Quantification of pathogen infection (qPCR) Obtain rootstock canes from mother blocks (Season 2) Repeat quantification of pathogen infection (qPCR) (Season 2) Obtain rootstock canes from mother blocks (Season 3) Repeat quantification of pathogen infection (qPCR) (Season 3)	2023-04-28
Survey of rootstock mother plant "heads" for incidence of trunk pathogens (not part of the original project proposal)	Obtain small wood samples from the "heads" of the same mother vines sampled in Objective 1 (Season 1) Conduct fungal isolation from "head" samples. Identification of fungal pathogens (morphology). Identification of fungal pathogens (molecular). Obtain wood samples from the "heads" (Season 2)	2023-01-20

	Repeat fungal isolations and identifications (Season 2)	
Parallel to the trunk pathogen survey, do physiological analyses (total starch, total phenolics, total minerals, water content) on rootstock canes in mother blocks	Obtain rootstock canes from mother blocks (Season 1) Conduct analyses on canes (Season 1) Obtain rootstock canes from mother blocks (Season 2) Repeat analyses on canes (Season 2) Obtain rootstock canes from mother blocks (Season 3) Repeat analyses on canes (Season 3)	2022-08-25
Parallel to the trunk pathogen survey, determine morphological dimensions (primary and secondary lengths, fresh and dry weight, thickness) of rootstock canes in mother blocks	Conduct morphological determinations on canes (Season 1) Repeat morphological determinations (Season 2) Repeat morphological determinations (Season 3)	2022-04-28
To link the trunk pathogen survey, physiological analyses and morphological dimension data with percentages take	Conduct a detached shoot assay by vacuum-inoculating rootstock shoots and determining the effect of pathogen inoculum on callus formation. One-year-old canes obtained from the most and least infected mother blocks identified during the survey will be grafted, planted and assessed once uplifted from nursery soils.	2022-07-03
Final Report	Finalise data and statistical analyses (March 2023) Final Report	2024-08-19

6. WORKPLAN (MATERIALS AND METHODS)

Phase 1: Survey of one-year-old canes from rootstock mother blocks over three seasons

1.1) A list of certified rootstock mother blocks that includes the most important cultivars/clones at different ages was compiled from information obtained from the Vine Improvement Association.

1.2) Fungal isolations were made from one-year-old canes. Recovered fungal isolates were identified based on colony morphology and by means of species-specific PCR and sequencing of representative isolates. Phylogenies were then constructed.

1.3) Quantitative PCR was done to determine the infection levels of *Phaeomoniella chlamydospora* and *Diplodia seriata*.

1.4) Parallel to the trunk pathogen survey, physiological analyses (total starch, total minerals, water content, total phenolic index) were also conducted to determine the overall health of the canes to be used for grafting.

1.5) Morphological dimensions (primary and secondary lengths, fresh weight, thickness) of rootstock canes in mother blocks were determined.

1.6) An additional survey was conducted on young and old block blocks where all canes from 50 plants of each block were removed, counted, and the thickness measured in order to determine the total yield and percentage of usable canes.

Phase 2: Survey of heads from rootstock mother blocks over two seasons

2.1) Heads of the same rootstock plants (mentioned above) were surveyed.

2.2) Fungal isolations were made from a cross-section of the head of each vine. Recovered fungal isolates were identified based on colony morphology and by means of species-specific PCR and sequencing of representative isolates. Phylogenies were then constructed.

2.3) Common occurring symptom types within the heads of the vines were linked to specific pathogens.

Phase 3: Determining the effect of pathogen inoculum on callus formation

3.1) A detached shoot assay was conducted to determine the effect of *Phaeomoniella chlamydospora* inoculum on callus formation of rootstock material.

Phase 4: Determining the effect of vine health on nursery vine performance

4.1) One-year-old canes from the most important cultivars/clones were harvested from vines that have been assessed (as described in phases 1 and 2) to include diseased rootstock material and healthy material as a control.

4.2) These canes were grafted with Chenin blanc scion cultivar in a commercial nursery setup, allowed to callus and rooted for one season according to standard nursery procedure.

4.3) The vines were uprooted, and the percentage of certifiable vines and root mass was determined. Fungal isolations were made from 25% of the vines.

4.4) Recovered fungal isolates were identified based on colony morphology.

7. RESULTS AND DISCUSSIONS

This first section of the study investigated the morphological and physiological status of one-year-old canes produced by grapevine rootstock mother blocks of different ages, in the Wellington region of South Africa. The morphological and physiological data over three seasons revealed trends between older and younger rootstock mother blocks only in certain instances, and within specific seasons. Furthermore, principal component analysis of the data of three seasons revealed planting year and season to have variable effects on the data for each rootstock variety, without conclusive association with block age. However, upon further investigation, correlations frequently occurred between certain morphological and physiological variables. When evaluating total canes yielded by blocks of different ages, it was found that, even though older blocks yielded more canes, a larger percentage of canes obtained from younger rootstock mother blocks fell within the diameter parameters for grafting as required by the grapevine industry.

The morphological and physiological data recorded over three seasons, including mineral content, starch content, TPI, water content, primary and secondary cane length, cane thickness and cane mass, were influenced by rootstock variety, season, and plant year. Rootstock mother block age was, therefore, considered inconsequential in this section of the study. The seasonal influences can be ascribed to environmental conditions, which are known to affect vine physiology (Bianchi and Brancadoro, 2021). Soil type, specifically soil mineral and water holding capacity, and fertilisation and irrigation regimes that differ from one season to the next, may have further impacted the data. Rootstock genotypes have also been noted to have a pronounced influence on mineral nutrition (Bianchi and Brancadoro, 2021). Disease incidence may further impact the morphological and physiological parameters, and was investigated later in this study.

In plant tissues, inorganic elements are mainly classified into macronutrients (N, S, P, Ca, Mg, K) and micronutrients (Ni, Mo, Cu, Zn, Mn, B, Fe, Cl) according to Marschner (2011). Nutrient availability and its impact on plants are influenced not only by the type of nutrient source but also by various soil factors like pH, redox potential, and microbial activity (Mimmo et al., 2014; Pii et al., 2015). Furthermore, the interaction between these nutrients can result in either antagonistic or synergistic effects that impact the overall efficiency of nutrient utilisation (Rietra et al., 2017). However, plant growth and development are influenced by nutrient interactions only when a specific nutrient is deficient in comparison to the nutrients applied (Fageria et al., 2014).

Taking three seasons' data into account, significant positive correlations were recorded between several minerals in three or more rootstock varieties in the current study (Figs. 1 – 9). For instance, B and Fe correlated positively in six rootstock varieties. Furthermore, B and Cu, and Mg and P correlated positively in five rootstock varieties. Similarly, Cu and Fe, Cu and Zn, and Mg and Mn correlated positively in four rootstock varieties, while B and K, B and Zn, K and Zn, and Mn and Na correlated positively in three rootstock varieties. In contrast, significant negative correlations were observed between K and Na in three rootstock varieties. Similar results were obtained by Rozane et al. (2020), who observed significant correlations between nutrients and coefficient of determination between Ca and Mg, P and S, N and S, N and P, P and Mg in grapevine, while numerous other studies reported synergies and antagonism between minerals in other plants (Aulakh and Malhi, 2005; Baxter et al., 2012; Pii et al., 2015; Toumi et al., 2016; Casanova-Gascón et al., 2018; Rozane et al., 2020; Xie et al., 2020). For instance, when B concentration is below toxic levels, increasing B concentration enhances Fe accumulation, but excessive B concentration reduces it (Long et al., 2023). This suggests that an optimal B level indirectly influences Fe absorption by stimulating plant growth and increasing Fe demand. Studies have shown that B aids in the long-distance transport of Fe from the roots to the aboveground parts by promoting the reuse of apoplast Fe (Long et al., 2023). These correlations between nutrients indicate that changes in the concentration of a given nutrient in the plant can consequently change the concentration of others (Parent et al., 2013; Rozane et al., 2015; Rozane et al., 2020). In addition to the correlations between nutrients and yield, the findings described above evidenced the need to use bi- or multivariate methods to diagnose nutritional status (Rozane et al., 2020). Most macronutrients have synergistic interactions, leading to one to three times higher yields than expected from individual nutrients (Rietra et al., 2017). Conversely, antagonisms and negative effects often involve divalent cations with similar uptake mechanisms (Rietra et al., 2017). In formulating effective fertilisers, minimising adverse nutrient interactions and maximising beneficial ones are crucial for optimal nutrient efficiency (Rietra et al., 2017).

Apart from the correlations observed between minerals, there were correlations between minerals and cane morphology (Fig. 1 – 9). These included a significant positive correlation between primary cane length and Cu in four rootstock varieties. This phenomenon could be incidental or an indirect consequence, considering Cu containing sprays are used to keep the plants healthy and thus allowing them to express better growth. Significant positive correlations were also observed between cane mass and B, Cu and Fe, each recorded in three rootstock varieties. Positive correlations were also observed between starch content and N, starch content and K, and between starch content and P. This may be expected as these minerals are essential elements for growth and development and thus would stimulate starch/reserve accumulation under non-stressed growth conditions. Nitrogen, K, and P are essential components of fertilisers and are frequently used to stimulate growth to replace minerals invested in canes that are lost when canes are harvested. The starch reserves stored in canes prior to propagation significantly influence grapevine growth, playing various roles in development, energy supply, and defence mechanisms against environmental stresses (Yang and Hori, 1979). Potassium is involved in the osmoregulation of stomatal cells, and together with Mg is involved in chlorophyll and protein (and thus RubisCO/photosynthesis) synthesis. Nitrogen forms part of proteins and nucleic acids and thus plays an integral role in plant structures, photosynthesis, metabolic pathways and the storage and translation of genetic information (Novoa and Loomis, 1981). Interestingly, water content correlated negatively with TPI, Cu and B in three rootstock varieties. Plants accumulate phenolics and switch from primary to secondary metabolism in response to various stressors, both biotic and abiotic (Lattanzio, 2013), likely explaining this phenomenon. However, it is important to note that even though overall TPI content yielded these results, it is important to note that these measurements may obscure ecologically significant variations in individual compounds (Langenheim, 1994; Keinänen et al., 1999; Martin et al., 2009).

Results from the survey of total one-year-old canes from rootstock mother blocks have shown that younger rootstock mother blocks produce more usable canes (6.5 - 12 mm) than older rootstock mother blocks, even though older blocks produced more canes overall (Table 1). This phenomenon can be ascribed to the fact that, with the higher yield of canes from older rootstock mother blocks, source partitioning to the higher number of sinks occurs, which ultimately results in thin canes which fall outside the industry's preferred parameters. Furthermore, large sections of white rot were observed at the top of ageing rootstock mother vine heads (refer to chapter 3), which is the section of the head often producing thicker canes in young rootstock mother vines.

In the next section, the infection levels of GTDs in grapevine rootstock mother blocks of different ages, and canes produced by them, in the Wellington region of South Africa are further presented. High incidences of GTD pathogens were recorded in the heads of all rootstock mother vines, mostly irrespective of age, with the main occurring family being Celotheliaceae. The main occurring symptom type within the heads was sectorial or undefined necrosis, often associated with Celotheliaceae. Much lower GTD incidences were recorded in one-year-old rootstock canes, with Botryosphaeriaceae being the predominant family isolated. qPCR analysis revealed incrementally higher positive samples in one-year-old canes than recorded with isolations, especially with regard to Pa. chlamydospora (Celotheliaceae).

Pathogens of all major GTDs were isolated from the heads of mother vines in certified rootstock mother blocks (Table 2). Incidences of up to 90.00% of Celotheliaceae were reported in heads of an eight-year-old block and occurred at 30.00% in a block as young as four-years-old. According to the literature internal symptoms that are associated with these pathogens include the formation of black exudates from infected vessels and brown to black streaking of the xylem (Mugnai et al., 1999; Rooney-Latham et al., 2005) that consists of tyloses and derivatives of phenolic compounds that have antifungal activity and is produced by the host in reaction to pathogen infection (Ferreira et al., 1994; Mugnai et al., 1999; Amalfitano et al., 2000; Lorena et al., 2001; Del Río et al., 2004) as well as brown necrosis that can be sectorial (Larignon and Dubos, 1997; Mugnai, 1999; Pollastro et al., 2000; Serra et al., 2000; Sofia et al., 2006; Calzarano and Di Marco, 2007; Péros et al., 2008; Gramaje et al., 2011; 2018; Bruez et al., 2014). Indeed Celotheliaceae were the main isolated fungal family from both black and brown wood streaking as well as sectorial or undefined necrosis, being present in 17.14% and 11.56% of all isolations made in the respective symptom types (Table 3, Fig. 10), which is consistent with results from previous studies (Larignon and Dubos, 1997; Mugnai et al., 1999; Pollastro et al., 2000; Sofia et al., 2006; Calzarano and Di Marco, 2007; Péros et al., 2008; Sánchez-Torres et al., 2008). The correlation observed between black and brown wood streaking and sectorial or undefined necrosis possibly shows wood streaking to directly result in sectorial or undefined necrosis, although not resulting in the diminish of the latter symptom (Fig. 11). The positive correlation observed between the Celotheliaceae and wood streaking shows that Pa. chlamydospora is the primary cause of this symptom type, as reported by numerous previous studies (Ferreira et al., 1994; Mugnai et al., 1999; Amalfitano et al., 2000; Lorena et al., 2001; Del Río et al., 2004).

The negative correlation observed between DNA concentration of Pa. chlamydospora and water content indicates that Pa. chlamydospora colonization are higher in water-stressed cane material (Fig. 11), or that the stress has been caused by the infection, as shown in the results obtained by Whiting et al. (2001). Physiological monitoring showed that sap flow density and whole-plant transpiration of Esca-infected grapevines decreased significantly a week before the first foliar symptoms appeared (Ouadi et al., 2019). Phaeomoniella chlamydospora's negative correlation with starch content in the one-year-old canes (Fig. 11) can be ascribed to this pathogen degrading the starches within the vines, as shown by Rolshausen et al. (2008), Fontaine et al. (2016) and Pouzoulet et al. (2017). The pathogen has long been known to produce the necessary enzymes for starch breakdown (Mugnai et al., 1999; Santos et al., 2006). Another possible explanation is that infection could have caused a decrease in growth and physiological functioning to such an extent that starch accumulation was neglected, especially if the pathogens caused vine stress. This phenomenon can also be ascribed to the activation of plant defence responses where carbohydrate metabolism plays an important role (Rojas et al., 2014), which is a cost-intensive mechanism (Berger et al., 2007). The result of these disruptions is a reduced reservoir of carbon reserves, which could potentially lead to a decline in plant growth and vitality in the following year (Fontaine et al., 2016).

Petri disease pathogens spread as aerial inocula (Larignon and Dubos, 2000; Eskalen and Gubler, 2001; Mostert et al., 2006b; Eskalen et al., 2007; Moyo et al., 2014; Fontaine et al., 2016; Halleen et al., 2020) originating from pycnidia and perithecia inside cracks, old pruning wounds and beneath the bark of grapevines (Edwards et al., 2001; Rooney-Latham et al., 2004; 2005; Eskalen et al., 2005a, 2005b; Baloyi et al., 2013, 2018). The spores are dispersed by arthropods, wind, and rain splashes (Chiarappa, 2000; Larignon and Dubos, 2000; Fourie and Halleen, 2004; Marchi et al., 2006; Retief et al., 2006; Moyo et al., 2014) and can infect via the many pruning wounds made on the heads of mother vines (Larignon and Dubos 2000; Whiteman et al., 2003; Christen et al., 2007; Edwards et al., 2007; Gramaje and Armengol, 2011; Gramaje et al., 2011; Makatini et al., 2014; Gramaje et al., 2018; Waite et al., 2018) that remain susceptible for 3 to 16 weeks (Larignon and Dubos, 2000; Eskalen et al., 2007; van Niekerk et al., 2011a; Makatini, 2014; Elena and Luque, 2016). Halleen et al. (2020) found the spores of Pa. chlamydospora and Pm. minimum to be present in all rootstock nurseries in South Africa that were evaluated in their study, and that spore release events occur during the week or within 4 weeks of rootstock cane harvesting in rootstock mother blocks. The reason for its abundance as aerial inoculum was probably linked to the occurrence of their fruiting bodies in Western Cape vineyards (Baloyi et al., 2013; 2016) and subsequently resulted in high levels of infection in rootstock mother blocks as observed in the current study. In numerous instances, Celotheliaceae were isolated more from the heads of older rootstock mother blocks in comparison to young rootstock mother blocks, likely due tothe build-up of infections due to the pruning wounds not being protected.

These fungi are also associated with the chronic form of Esca and are considered by some as the pioneering pathogens of the disease (Mugnai et al., 1996; Larignon and Dubos, 1997; Mostert et al., 2006b; Gramaje et al., 2009), predisposing the vines to infection by fungi in the Hymenochaetaceae (Larignon and Dubos, 1997; Mugnai et al., 1999; Fischer, 2006; Cloete et al., 2015a; Gramaje et al., 2018). In general, the Hymenochaetaceae were more consistently isolated from older rootstock mother blocks (Table 2), which is in accordance with results obtained by previous studies (White et al., 2011a). However, species in the Hymenochaetaceae were isolated from rootstock mother blocks as young as three-years-old (Paulsen 1103 PS28I block with 8.00% incidence in the 2019 season).

White rot is typically associated with fungi in the Hymenochaetaceae and can be bordered by black lines and brown margins of decayed wood (Larignon and Dubos, 1997; Mugnai, 1999; Pollastro et al., 2000; Serra et al., 2000; Sofia et al., 2006; Calzarano and Di Marco, 2007; Péros et al., 2008; White et al., 2011a; Gramaje et al., 2011; Bruez et al., 2014). In the current study, 30.78% of all isolations made from white rot yielded species of Hymenochaetaceae, while 21.80% of the isolations made from necrosis or lines in or adjacent to white rot yielded species of Hymenochaetaceae (Table 3). These results are reflected in the multiple factor analysis that showed the Hymenochaetaceae to be mainly associated with these two symptom types (Fig. 11). The Hymenochaetaceae was not only limited to white rot and necrosis or lines in and adjacent to white rot, but was also isolated from sectorial or undefined necrosis, black and brown wood streaking and black or brown margins adjacent to necrotic wood (Table 3). This is consistent with a report by Sparapano et al. (2001), who showed that Hymenochaetaceae can induce brown discolouration in grapevine wood during the initial stages of colonisation.

The negative correlation observed between white rot and necrosis in or adjacent to white rot, and black and brown wood streaking (Fig. 11) likely shows that wood streaking, which correlated positively with Celotheliaceae, is the initial phase of infection and diminishes once white rot, caused by Hymenochaetaceae and Togniniaceae, establishes. This can be ascribed to an observation made by Bruez et al. (2020), who found a decrease in the diversity of fungal communities in the white rot of diseased trunks, where F. mediterranea becomes predominant. While many studies have shown F. mediterranea to be the main causal agent of white rot of grapevine in Europe (Larignon and Dubos, 1997; Mugnai et al., 1999; Pollastro et al., 2000; Sofia et al., 2006; Calzarano and Di Marco, 2007; Péros et al., 2008; Sánchez-Torres et al., 2008; Bruno et al., 2021), the predominant species in the current study conducted in South Africa were F. capensis and an undescribed Fomitiporella species (Taxon 1). Togniniaceae, having a positive correlation with margins adjacent to necrotic wood and necrosis in or adjacent to white rot, likely spreads from margins adjacent to necrotic wood and contributes to white rot and necrosis in or adjacent to white rot and is, therefore, present within the heads before the arrival of the Hymenochaetaceae. The correlation observed between the incidence of Hymenochaetaceae and Togniniaceae (Fig. 11) and necrosis or lines adjacent to white rot in the heads of rootstock mother vines indicates that these two fungal families co-occur within this symptom type.

The results with regard to Esca-associated pathogens presented here are of critical importance, as these pathogens can significantly reduce the lifespan of a mother vine. A study by Liminana et al. (2009) ,who investigated Esca in rootstock mother vines have shown the necrotic area percentage to correlate significantly with vine mortality and white rot incidence. Moreover, the negative correlation between white rot in the heads of mother vines and primary length of one-year-old canes, as observed in the multiple factor analysis (Fig. 11), indicated that white rot, caused by Hymenochaetaceae, results in shorter canes being produced and thus has a significant negative impact on rootstock mother block production.

In the current study, six basidiocarps, belonging to Basidiomycete Taxon 1, Taxon 3 and Fomitiporia capensis, were found in certified rootstock mother blocks between the ages of nine and 13 years old (Fig. 12). These basidiocarps produce basidiospores that can infect grapevines through susceptible pruning wounds (Cortesi et al., 2000; Graniti et al., 2000; Surico et al., 2000; Fischer, 2002).

The causal agents of Botryosphaeria dieback were the second most isolated pathogens from rootstock mother vine heads in the current study. The predominant identified species were D. seriata, while N. parvum, D. mutila, L. plurivora, N. australe, D. pterocarpi, L. mahajangana and N. vitifusiforme occurred to a lesser extent. These results differ from those obtained in previous reports in South Africa, where N. australe and N. stellenboschiana have been shown as the major Botryosphaeriaceae pathogens in commercial vineyards (van Niekerk et al., 2004; Sieberhagen, 2016; Mojeremane et al., 2020). Internal manifestations associated with the disease include perennial cankers, trunk dieback, characteristic wedge-shaped necrosis and vascular streaking (Úrbez-Torres, 2011; Fontaine et al., 2016; Gramaje et al., 2018; Billones-Baaijens and Savocchia, 2019; Hrycan et al., 2020; Reis et al., 2020) or circular to nonuniform central staining of the wood observed in cross-sections (Gramaje et al., 2018). In the current study Botryosphaeriaceae was the main isolated pathogenic family in the black and brown margins adjacent to necrotic wood occurring in 12.47% of all isolations made from this symptom type, while being the second most isolated family in both the black and brown wood streaking as well as sectorial and undefined necrosis, at 3.87% and 10.43%, respectively (Table 3). According to the literature, symptoms are usually observed in vineyards aged 8 years and above (Úrbez-Torres, 2011), although cankers, dieback, and plant death have been recorded in 3- to 5-year-old table grape vines (Úrbez-Torres et al., 2008), as observed in this study.

Whitelaw-Weckert et al. (2013) isolated Botryosphaeriaceae species, predominantly D. seriata, from 95% of rootstock nursery vines. In the current study, incidences as high as 42.00% have been reported in a three-year-old rootstock mother block, while incidences as high as 84.00% have been recorded in an 11-year-old block (Table 2). Apart from the high incidences of Botryosphaeriaceae recorded in older blocks, a recent study by Larach et al. (2023) has also shown D. seriata to be more virulent in older wood compared to young tissue, which further predisposes the vines to infections by pathogens of the GTD complex.

Botryosphaeriaceae produce pycnidia and pseudothecia on decayed wood or pruned remnants, releasing pycnidiospores and ascospores when wet, rainy conditions prevail (Phillips et al., 1998; Úrbez-Torres et al., 2010; van Niekerk et al., 2010; Fontaine et al., 2016; Gramaje et al., 2018). These spores are dispersed through wind or rain-induced splashing (Gubler et al., 2005; Úrbez-Torres et al., 2010) and ultimately infect susceptible pruning wounds (van Niekerk et al., 2010; Úrbez-Torres et al., 2011; Kotze et al., 2011; Havenga et al., 2019). Botryosphaeriaceae spores were trapped from late-autumn to early spring in South Africa (van Niekerk et al. 2010), which coincides with the harvesting period of canes from rootstock mother vines. Contaminated pruning tools have also been shown to spread infection (Epstein et al., 2008; Agustí-Brisach et al., 2015). This is of major importance as pruning tools are not frequently sterilised when the canes are harvested in rootstock mother blocks in South Africa. Additionally, research has demonstrated that these fungi have the capability to infect healthy plants directly through lenticels, stomata, or other openings (Brown et al., 1981; Michailides et al., 1991), facilitated by the presence of inocula on the bark of rootstock cuttings obtained from the mother vine (Billones-Baaijens et al., 2015).

The negative correlation observed between the incidence of Botryosphaeriaceae and specific minerals, as observed in the multiple factor analysis (Fig. 11), likely shows the growth of these pathogens needing, or depleting, these specific minerals within the vines. Moreover, its negative correlation with cane morphology shows that it directly impacts the development of one-year-old canes. This can likely be ascribed to fungal metabolites being produced by these fungi that result in structural modifications of the canes, specifically that of suberin deposition (Fleurat-Lessard et al., 2013) or simply because they restrict optimal shoot growth because of the interference with natural physiological mechanisms.

The necrosis or lines in or adjacent to white rot that correlated positively with Botryosphaeriaceae incidence in the heads of mother vines (Fig. 11) show that species in the

Botryosphaeriaceae co-occur in Esca-associated symptoms and likely have a synergistic effect. Members of the Botryosphaeriaceae family are known to be associated with esca diseased vines (Mugnai et al., 1999), although their specific role remains uncertain (Calzarano and Di Marco, 2007). In the past, these fungi have been isolated from various esca-related symptoms, with a higher frequency in cases of sectorial necrosis (van Niekerk et al., 2006; Larignon and Dubos, 1997; Mugnai et al., 1999). Multiple trunk pathogens and other wood-colonising fungi may interact in the process of wood decomposition (Sparapano et al., 2000a). This can result in facilitative interactions among them (Travadon et al., 2016), which is more likely in communities harbouring more species. Furthermore, in the presence of multiple GTD fungi within a grapevine, this threshold for resource utilisation could be reached more quickly (Hrycan et al., 2020).

Phenolic compounds, such as tannins, phenolic acids, flavanols and stilbenoids, play a significant role in the defence mechanisms of vines, enhancing the plant's resistance (Del Rio et al., 2004). The initial stage of the defence response to infection involves the emergence of polyphenol-rich reaction zones at the site of infection and accumulation of phenolic compounds in the xylem vessels, effectively limiting the development and spread of fungal hyphae (Del Rio et al., 2001; Agrelli et al., 2009; Fontaine et al., 2016). However, Martin et al. (2009) found that grapevine tissue types showed variation in phenolic accumulation, with the greatest accumulation observed in young shoots compared to the trunk. The positive correlation between the TPI in one-year-old canes and the incidence of Botryosphaeriaceae and Hymenochaetaceae in the heads of rootstock mother vines (Fig. 11) suggests that infection by these Esca-associated pathogens results in a more severe reaction, likely due to a locally induced response. Indeed, Wallis et al. (2022) found D. seriata to be associated with significantly higher concentrations of phenolic compounds, while this species was shown to display high sensitivity to piceatannol, pterostilbene, and ε-viniferin (Lambert et al., 2012).

Species of Diaporthaceae, the causal agents of Phomopsis dieback, were isolated to a lesser extent from the heads of rootstock mother vines (Table 2). The species mainly consisted of D. founiculia and D. ampelina, while D. novem and D. ambigua occurred at lower levels. Diaporthe ampelina is historically the most common and virulent species known to cause Phomopsis cane and leaf spot, cane bleaching and grapevine swelling arm (Pscheidt and Pearson, 1989; Rehner and Uecker, 1994; Kajitani and Kanematsu, 2000; Mostert et al., 2001, van Niekerk et al., 2005; Baumgartner et al., 2013; Urbez-Torres et al., 2013a; Dissanayake et al., 2015; Guarnaccia et al., 2018) and has been shown to be a more aggressive colonizer that causes greater levels of wood discoloration in greenhouse assays than D. foeniculina (Lawrence et al., 2015). Despite Mostert et al. (2001) reporting 10 Diaporthe species on grapevines in South Africa, and van Niekerk et al. (2005) reporting 15 Diaporthe species, only five species have been found in grapevine rootstock mother blocks in the current study. Species of Diaporthaceae occurred mainly in sectorial or undefined necrosis and black and brown wood streaking (Table 3), which is in accordance with previous studies that associated these pathogens with perennial trunk cankers (Úrbez-Torres et al., 2013a; Guarnaccia et al., 2018; del Martínez-Diz et al., 2019). The Diaporthaceae incidence correlating negatively with black and brown necrosis or lines in or adjacent to white rot, white rot, Botryosphaeriaceae- and Hymenochaetaceae incidence (Fig. 11), shows that this family of pathogens are likely suppressed during the development of Esca, where Botryosphaeriaceae correlating positively with necrosis in or adjacent to white rot, and the Hymenochaetaceae that correlates positively with the latter symptom type and white rot, becomes the predominant pathogens within the heads of rootstock mother vines. This phenomenon can again be ascribed to an observation made by Bruez et al. (2020), who found a decrease in fungal communities during the progression of white rot.

The causal agents of Eutypa dieback, consisting of species belonging to the Diatrypaceae, were least isolated from grapevine rootstock mother blocks (Table 2). Only two species, namely E.

lata and Eu. citricola have been identified in this study, only comprising 0.9% of the total isolated pathogens. Despite being isolated at low incidences, the occurrence of E. lata in rootstock mother blocks is an important finding as it is one of the most virulent species contributing to Eutypa dieback (Carter 1991, Moyo et al., 2018). The majority of the Diatrypaceae were isolated from sectorial or undefined necrosis, followed by black and brown wood streaking (Table 3). This is in agreement with results obtained by White et al. (2011), who isolated E. lata at very low incidences, and predominantly from the sectorial brown necrosis. The positive correlation observed between the Diatrypaceae and Togniniaceae likely shows another synergistic occurrence (Fig. 11). The negative correlation between Diatrypaceae and cane morphology indicates that colonisation by this pathogenic family has a negative impact on cane development (Fig. 11).

The interplay among various fungal species within diseased grapevine wood, their respective roles in wood deterioration, their mutual interactions, and the toxins generated by these diverse species are believed to collectively influence the manifestation of symptoms (Mugnai et al., 1999; Surico et al., 2000; Surico et al., 2006; Calzarano and Di Marco, 2007).

Much lower incidences of GTDs were recorded following fungal isolations from one-year-old rootstock canes (Table 4). Rootstock mother block age correlated poorly with pathogen incidence in one-year-old canes. The highest total pathogen incidence was recorded in a 10-year-old rootstock mother block with an incidence of 32.00%, with 28.00% of that attributed to Botryosphaeriaceae infection (Table 4). The majority of the fungal pathogens belonged to Botryosphaeriaceae and were mainly identified as D. seriata. Therefore, it was decided to quantify the total DNA in canes from all blocks over three seasons using qPCR analysis. Diplodia seriata DNA concentrations as high as 3171 ng.µL-1 were recorded in the canes and occurred in the majority of rootstock canes (Table 5), similar to the results obtained by Spagnolo et al. (2011). Fourie and Halleen (2002) suggested that Botryosphaeriaceae species contaminated one-year-old canes from infected mother vine heads, as higher incidences of the pathogens were found in the basal internodes of one-year-old rootstock canes. This can be ascribed to mycelial growth or via conidia being transported via sap flow through xylem vessels (Pascoe and Cottral, 2000; Feliciano and Gubler, 2001; Fourie and Halleen, 2002; Edwards et al., 2004; Whiteman et al., 2005, 2007). In 2015, Billones-Baaijens et al. suggested dormant cutting bark as a potential infection source for Botryosphaeriaceae species. In 2018, Waite et al. confirmed the epiphytic inoculum's ability to penetrate cane bark and cause infections.

Despite Pa. chlamydospora occurring at low incidences in rootstock canes when recorded using traditional isolation methods, its DNA was quantified in canes from all blocks over three seasons using qPCR analysis, as it was the main isolated pathogen from the heads of rootstock mother vine heads (Table 5). Moreover, results by Elena et al. (2016b) showed recovery of D. seriata from canes to be easier compared with Pa. chlamydospora. The majority of canes were considered incidence-free when determining infection levels of Pa. chlamydospora using isolation methods (Table 4), similar to the finding by Zanzotto et al. (2001), who were unable to successfully isolate Pa. chlamydospora from suspected infected cutting material, and Fourie and Halleen (2002), who managed to isolate the pathogen, albeit in very limited quantities. In contrast to these findings, with the exception of one block’s canes in one season, all pooled samples from all rootstock mother blocks over all three seasons contained detectable levels of Pa. chlamydospora DNA (Table 5). The highest Pa. chlamydospora DNA concentration was in healthy canes of a relatively young rootstock mother block, at the age of seven years old, with a concentration of 1055.25 ng.µL-1 Table 5). Bruez et al. (2020) reported Pa. chlamydospora to be the most abundant species in non-necrotic tissues from healthy plants, suggesting a possible non-pathogenic endophytic behaviour.

In 2007, Whiteman et al. found that Pa. chlamydospora could move from the retained rootstock mother-vine head to the current season's growth. They observed that vines from dormant cuttings taken closest to the head had a higher infection rate of 42% after 8 months, while those from further away exhibited infection rates of 6-8%. In the current study, qPCR analysis was done on plant material from the 3rd internode from the basal end of the canes, which is likely a contributing factor in the high DNA concentrations obtained in the current study.

The negative correlations observed between the incidence of Pa. chlamydospora and DNA concentrations thereof in one-year-old canes (Fig. 13) can likely be ascribed to inoculum nestled beneath the bark of the canes, which is killed during the process of surface sterilisation when doing traditional fungal isolations. The presence of Pa. chlamydospora has been observed beneath the bark where injury resulted in exposed vascular tissue of grapevines (Edwards et al., 2001). This external inoculum may be introduced to sprawled canes directly from infested soils (Retief et al., 2006; Whiteman et al., 2007; Sacca et al., 2018) as reported for bark at the soil level (Rooney-Latham et al., 2001). The negative correlations may also show the difficulty of isolating this species from wood, even though DNA in the form of mycelia or spores are present within the canes. Several studies have detected spores and hyphal fragments of Pa. chlamydospora along the length of rootstock canes (Feliciano and Gubler, 2001; Edwards et al., 2003; Retief et al., 2005). Low concentrations of fungal DNA contained in these forms may be easily detected using qPCR, as total DNA is extracted from 10 internodes, while traditional isolation methods rely on the isolation of these fragments from small bundles of xylem vessels. A similar observation has been made in the past, where Retief et al. (2005) found up to four times more positive detections with qPCR when compared to traditional isolation techniques. Luo et al. (2016) showed qPCR assays to be more efficient, accurate and precise to quantify latent infections caused by canker-causing pathogens as compared to the traditional plating methods. These results indicate that one-year-old canes from rootstock mother blocks can harbour GTD inoculum, either systemically or as inoculum underneath the bark, which serve as a source of infection in both the grapevine nurseries as well as in newly established vineyards.

With regards to D. seriata, the positive correlation observed between its incidences and DNA concentration in one-year-old canes and incidences in the heads of rootstock mother vines (Fig. 13) indicates that higher levels of infection in the heads do indeed result in more contaminated one-year-old canes, likely due to the movement of spores within canes or as active mycelial growth from the heads of infected mother vines as described by Fourie and Halleen (2004). Similar findings were raised by Amponsah et al. (2012), who reported that hyphae inthe xylem of grapevine stems inoculated with species of Botryosphaeriaceae moved faster acropetally than basipetally. The authors reisolated N. luteum 60 - 70 cm acropetally from the inoculation site after 4 months, by which time the pathogen had progressed into side shoots. The correlation between incidences and DNA concentration in one-year-old canes shows that traditional fungal isolation methods for this pathogen give a more accurate representation of the inoculum levels, in comparison to those of Pa. chlamydospora. This can likely be ascribed to inoculum beneath the bark that penetrates healthy plant tissue and colonises the phloem vessels as described by Billones-Baaijens et al. (2015) and can, therefore, be isolated using traditional isolation techniques, even though the external inoculum is killed during the process, while the same might not be true for Pa. chlamydospora. In a study by Billones-Baaijens et al. (2013), who investigated three commercial nurseries, 33% to 100% of canes contained detectable amounts of Botryosphaeriaceae on the surfaces of the canes, and 15% to 68% of canes were internally infected with these fungi, while Whitelaw-Weckert et al. (2013) isolated D. seriata from 25% of canes sampled from the rootstock mother blocks.

A noteworthy observation is the positive correlation observed between the incidences of Pa. chlamydospora and D. seriata in the heads of rootstock mother vines (Fig. 13), which may suggest another synergistic infection. Spagnolo et al. (2011) and Elana et al. (2016b) reported infections by Botryosphaeriaceae and Pa. chlamydospora to frequently co-occur in propagation material, albeit with a slower colonisation rate by Pa. chlamydospora than D. seriata (Elana et al., 2016b). Both Botryosphaeriaceae species and Pa. chlamydospora have been shown to produce the phytotoxic metabolites iso-sclerone and exopolysaccharides (Sparapano et al., 2000b; Martos et al., 2008; Evidente et al., 2010), and could result in a synergistic action (Spagnolo et al., 2011).

The results of this section of the study correspond to those obtained by Fourie and Halleen (2004), who surveyed grapevine rootstock mother plants from different ages, different cultivars, and different geographical areas in order to determine the frequency of occurrence of grapevine trunk disease pathogens. The authors found that vine age and the incidence of these fungi correlated poorly, indicating that increased infection and colonisation over time in the asymptomatic rootstock mother plants, as was expected, was not observed. Despite not being able to properly differentiate between rootstock mother blocks of different ages, the research contained in this study sheds light on the critical condition of South Africa's grapevine rootstock mother blocks and shows the urgent need for proper management of these blocks. Such management strategies should include the use of pruning wound protectants throughout the lifespan of rootstock mother blocks to prevent infection of pruning wounds by aerial inoculum of GTDs. Frequent renewal of the head could also reduce the build-up of fungal infections (Smart et al., 2023). This can be done by raising the head from ground level as currently practised. In fact, trellising of the rootstock canes should be investigated as proposed by Smart et al. (2023) to reduce the negative impact of GTDs on the quality of propagation material. Sanitation should also be applied, where pruning remnants, fruiting bodies and dead vines are removed from rootstock mother blocks on a regular basis. This research also showed the negative effect of GTD infections on cane production in rootstock mother blocks. One-year-old canes were shown as a primary source of inoculum for Pa. chlamydospora and D. seriata. The results validate the complementary use of molecular tools along with the classical methods in the diagnosis and detection of D. seriata and Pa. chlamydospora and enable their detection in asymptomatic wood, as in the propagation material.

This last phase investigated the effect of Pa. chlamydospora inoculum on rootstock basal callus formation and the effect of cane health on nursery vine performance. Phaeomoniella chlamydospora was shown to inhibit callus formation. Low incidences of GTDs were recorded in rootstock material before grafting, irrespective of mother block age, while higher incidences were recorded in grafted nursery vines following uprooting in the nursery. In many cases, the rootstock clone, age of rootstock mother block from which plant materials were collected, and position of isolations from the grafted nursery vines had an influence on GTD incidence. Neither the age nor the clone of the rootstock mother blocks had an effect on the percentage certifiable vines. While both had an effect on root mass, no clear trends were observed between vines grafted from young versus older rootstock mother blocks.

Results from the field trial evaluating the effect of rootstock cane health nursery vine performance indicated that scion material was free of GTD infections (results not shown), showing rootstock mother vines and surface inoculum as the only source of infection before the nursery process. These results are in agreement with those obtained by Bertelli et al. (1998), Sidoti et al. (2000), Zanzotto et al. (2001), Halleen et al. (2003) and Aroca and Raposo (2005). Low incidences of Botryosphaeriaceae and Pa. chlamydospora were recorded in rootstock material prior to grafting, but did not differ significantly from pathogen incidence-free material. Following the uprooting of the grafted nursery vines after 7 months in the field, higher incidences of GTDs were recorded than before grafting. Similar observations were made by Fourie and Halleen (2002), who found low levels of Pa. chlamydospora and Phaeoacremonium spp. in rootstock material following isolations after grafting and callusing, while significantly higher levels were observed when isolations were done from grafted nursery vines after uprooting in the nursery.

Decades of using the same nursery soil in South Africa, with a 2-year rotation system for planting cuttings alternated with cover crops, have resulted in an increase in soil-borne inoculum of black foot pathogens (Halleen et al., 2003). It is thus not surprising that the majority of black foot pathogens were recorded in the basal end of grafted nursery vines (Table 6). These results are in accordance with reports by previous studies that showed black foot pathogens to infect through the basal end where the pith is exposed to soil-borne inoculum, as callus tissue does not typically cover the entire area (Halleen et al., 2003, 2006; Agustí-Brisach et al., 2013). The incidence of black foot pathogens in the graft union can be ascribed to infection of the graft union by soil-borne inoculum while the vines are covered in soil during the five weeks after planting, before the soil is removed around the scion, graft union and upper part of the rootstock. This practice is followed by South African nurseries to avoid drying out of the graft union (Halleen et al., 2006). Interestingly, black foot pathogens were isolated significantly more from nursery vines grafted with rootstock material originating from older rootstock mother blocks (Table 7).

The majority of Botryosphaeriaceae were isolated from the graft unions of grafted nursery vines, and to a lesser extent from the basal ends (Table 8). This phenomenon might be ascribed to the infection of the graft unions by Botryosphaeriaceae inoculum that is latent in dormant cutting bark, as suggested by Billones-Baaijens et al. (2015) or from infection or contamination during the grafting process. No clear trends were observed between plots grafted from plant material sourced from rootstock mother blocks with different health statuses or clones. However, all graft unions and basal ends of grafted nursery vines of Richter 99 RY13C were incidence-free (Table 8). Similarly, Sieberhagen (2016) found Richter 99 RY13C to have lower disease severity than other major rootstock varieties in South Africa after inoculations with various GTD pathogens. The positive association observed between D. seriata DNA concentration in canes before grafting and incidence in the graft union after uprooting (Fig. 14) suggests that the inoculum of this pathogen establishes and increases during the nursery phase.

Phaeoacremonium species were mainly isolated from the graft unions of vines grafted from the older rootstock mother blocks (Table 9). The incidences also varied between varieties, with Ramsey having the highest level of infection, followed by Paulsen 1103 PS28I and US 8-7 UC274A and Ramsey SC18AB (Table 10). Diaporthe spp. incidence was recorded only in three rootstock varieties (Table 10). The highest infection occurred in US 8-7 UC274A, followed by Paulsen 1103 PS28I and Ramsey SC18AB (Table 11). The rest of the varieties were incidence-free. These phenomena can be ascribed to the susceptibility of rootstocks that vary to different GTDs (Eskalen et al., 2001; Feliciano et al., 2004; Gramaje et al., 2010; Billones-Baaijens et al., 2014; Murolo et al., 2014; Martínez-Diz et al., 2019).

Pleurostoma richardsiae was not isolated from the rootstock mother vines or scion material before grafting, but was the predominant pathogen occurring in grafted nursery vines following uprooting in the grapevine nursery. In the past, the fungus has frequently been isolated from young diseased vines, specifically from around the pith areas in graft unions (Halleen and Groenewald, 2005), which is in accordance with the results obtained in the current study. Although not always statistically significant, Pl. richardsiae occurred at higher incidences in older blocks of five of the seven varieties, while no infections were recorded in either block of Richter 99 RY13C (Table 12). The positive association between root mass and Pl. richardsiae incidence (Fig. 14) can be ascribed to more water and nutrient availability in the graftlings, making it easier for the pathogen to colonise graftlings once planted in the nursery. From these results, it is clear that Pl. richardsiae is introduced during the grapevine propagation process, either during grafting, callusing or rooting in the nursery field. Measures to control infection by this pathogen, therefore, need to be incorporated in the nursery process.

Phaeomoniella chlamydospora was able to inhibit the formation of basal callus in Paulsen 1103

PS28I, with an EC50 concentration ranging between 105 and 208 conidia mL-1 in the detached shoot assay (Table 13, Fig.15). These results are in agreement with those obtained in previous studies (Santos et al., 2005, 2006; Diaz et al., 2009). This phenomenon can be ascribed to the production of phytotoxic metabolites such as exopolysaccharides by Pa. chlamydospora. The adverse effect of this pathogen was further observed in the field trial, where DNA concentration of Pa. chlamydospora in one-year-old canes and wet root mass of grafted nursery vines following uprooting correlated negatively (Fig. 14), suggesting that Pa. chlamydospora negatively impacts root development during the nursery phase, again likely due to the production of phytotoxic metabolites. These results contradict those obtained by Wallace et al. (2004), who found that root initiation was not inhibited by Pa. chlamydospora. However, these authors inoculateda low concentration of conidia and evaluated root formation after only 10 weeks in the nursery. Moreover, in order to trigger a response from the host, the vine must employ resources and nutrients to effectively isolate the fungi within the plant, leading to the partitioning of resources that hinders plant growth (Grossman and De Jong, 1994). The lower pool of reserves might contribute to a significant decrease in plant growth (Petit et al., 2006; Fontaine et al., 2016).

While Pa. chlamydospora present in the planting material before grafting exhibits an inhibitory effect towards the growth of certain GTD pathogens, positive associations were observed between the incidences of numerous GTD pathogens, including Pa. chlamydospora, in grafted nursery vines following uprooting from the nursery fields (Fig. 14). Infection by certain pathogens may predispose the graftlings to infection by other pathogens. These positive associations may also be ascribed to the co-occurrence of these pathogens in weakly grafted nursery vines that are more prone to infection.

According to the authors' knowledge, this is the first study linking the vine nutrient status of mother block canes to GTD occurrence. The positive association observed between nitrogen in one-year-old canes and root mass (Fig. 14) indicates a direct growth effect from the stored nitrogen on nursery vines. The nitrogen reserves correlated positively with the incidences of certain GTD pathogens in the basal end of grafted nursery vines (Fig. 14), including species of Botryosphaeriaceae, black foot and Diaporthe (Fig. 14). Higher levels of zinc were associated with higher incidences of Botryosphaeriaceae and Diaporthe. Other minerals, including potassium, magnesium and boron, reduced the incidence of some of the GTDs (Fig. 14).

Rootstock mother block age, irrespective of health status, was shown not to have major implications on nursery vine performance. However, it was variety and pathogen-dependent. However, inoculum in contaminated canes originating from infected rootstock mother blocks has been shown to adversely affect nursery vine performance. Grapevine trunk disease infection has been shown to increase during the nursery process, especially with regard to Pl. richardsiae and black foot pathogens, where the nursery processes are the only source of infection. Managing grapevine trunk disease pathogens during the propagation process requires an integrated approach starting from the establishment of healthy rootstock mother blocks, preventing pruning wound infections in rootstock and scion mother blocks, preventing infections of harvested shoots during storage, grafting, callusing and establishment in vine nurseries as well as procedures (like hot water treatment) to eliminate pathogen infections once nursery vines are uprooted.

8. CONCLUSIONS AND RECOMMENDATIONS

The risks of rootstock mother vines becoming infected with grapevine trunk diseases (GTDs) such as Petri disease, Esca, Botryosphaeria dieback, Phomopsis dieback and Eutypa dieback, and supplying lower yields of propagation material with compromised phytosanitary status could seriously impact the success of the grapevine industry. It is unclear at which age rootstock mother vines need replacements in order to mitigate these risks; therefore, the aim of this study was to identify factors that may shorten the productive lifespan of grapevine rootstock mother blocks. Identification of such factors can increase the competitive edge of the South African grapevine industry, as it might make a positive contribution towards higher take percentages in nurseries. Most importantly, it may improve the phytosanitary status of grafted vines, which may lead to better establishment success and healthier vines with a longer productive lifespan. This study, for the first time, provided an opportunity to combine physical, physiological and GTD details on the same rootstock mother material and to provide answers that are hitherto unknown.

High incidences of GTD pathogens were recorded in the heads of all rootstock mother vines, mostly irrespective of age. This is of great concern, as this study has shown infected rootstock mother vines to contaminate one-year-old canes and serve as an inoculum source in the grapevine propagation process. The inoculum load within one-year-old canes did not correlate with rootstock mother block age. This observation can likely be ascribed to the high incidences of GTDs within young rootstock mother blocks, resulting in canes with similar inoculum levels to those obtained from older rootstock mother blocks. For instance, Hymenochaetaceae were isolated from rootstock mother blocks as young as three-years-old in this study. It was found that *Diplodia seriata* infection in the heads results in infected one-year-old canes, while the same was not true for *Pa. chlamydospora*. This research indicated that the external inoculum of *Pa. chlamydospora* may play a role in contamination of one-year-old canes. This form of contamination will not easily be managed due to the close proximity of rootstock mother blocks to vineyards and alternative hosts of GTDs. If infection is suspected, cuttings should be treated by soaking in a suitable fungicide solution or by hot water treatment (HWT) prior to grafting (Smart et al., 2023). However, benzimidazole fungicides like benomyl and carbendazim often used in the process, are in the process of being removed from international markets, and replacement products must be sought.

In the current study, Basidiocarps belonging to Basidiomycete Taxon 1, Taxon 3 and *Fomitiporia capensis* were found in certified rootstock mother blocks. Aerial inoculum from within rootstock mother blocks should be contained by means of sanitation (Waite et al., 2014; Elena and Luque, 2016a). This entails the removal and destruction of any dead plants or plant material, the removal of fungal fruiting bodies and the control of insect vectors. The spread of infections between mother vines can further be minimised by the regular sanitation of pruning shears during the pruning process. The use of pruning wound protectors is urged to be used by the South African grapevine industry immediately after pruning of rootstock mother blocks. Whether painting or spraying, it is crucial to ensure comprehensive coverage of all wounds, including those on one-year-old wood, regardless of their size (Smart et al., 2023). By protecting the susceptible pruning wounds on rootstock mother vine heads, infections by aerial inoculum of GTDs from within rootstock mother blocks or surrounding vineyards will be effectively managed and will aid in the production of healthier rootstock canes.

The numerous incisions on short shoots created during cane harvesting, coupled with the close proximity of the crowns to the soil surface (typically within 500 mm), render rootstock mother vines especially susceptible to infection by GTDs (Waite et al., 2018). Focussed pruning methods have been found effective in preserving vital elements of wood structures. Elena and Luque (2016b) suggested that the colonisation of GTD pathogens can be influenced by the length of stem internodes that remain after pruning. Consequently, it is advisable to trim each cane in a manner that preserves the longest internodes at the head. Faúndez-López et al. (2021) found that pruning cuts over nodes can protect basal buds and prevent wood necrosis. Bruez et al. (2022) demonstrated that maintaining 2 - 3 cm pruning spurs can halt desiccation and ensure unaffected sap flow. Similarly, Falsini et al. (2023) found that pruning treatments where short spurs were left exhibited fewer desiccation areas compared to pruning treatments without short spurs. This practice aims to minimise the risk of GTDs penetrating the heads, as suggested by Amponsah et al. (2012).

When evaluating rootstock cane morphology and physiology, the results revealed planting year and season to have variable effects on the data for each rootstock variety, without conclusive correlation with block age. The results indicate that, when adequate rootstock canes that meet these requirements are selected for propagation material, the physiological and morphological parameters will not be negatively affected based on rootstock mother block age. This data further suggests that physiological status is more affected by the season and fertilising and irrigation strategies than by vine age. It is crucial that the appropriate fertilising regime be implemented, and drought stress be avoided in order to produce canes with optimal starch and mineral contents. With proper management of ageing rootstock mother blocks, including proper disease management, sufficient fertilisation and irrigation, cane physiology and morphology will likely not impact grafting success.

Results from this study further revealed that, even though older blocks yielded more canes, a larger percentage of canes obtained from younger rootstock mother blocks fell within the diameter parameters for grafting as required by the grapevine industry. This finding is of great importance to the South African grapevine industry as it shows mother block replacements will result in higher yields of usable one-year-old canes. The phenomenon can likely be ascribed to older rootstock mother vines being heavily infected by all major GTDs, emphasising a continuous focus on the health care of rootstock mother blocks. This results in the gradual death of plant tissue at the top of the mother vine heads, as evident from the wide variety of symptoms, mostly on the upper part of the heads. Recent studies have also found wood age to have an impact on pathogen susceptibility, with older wood, such as that found in the heads of rootstock mother vines, being more susceptible to infection (Larach et al., 2023). This may result in the production of thin, and thus unusable, canes from the upper part of the mother vine head. Indeed, this study showed Botryosphaeriaceae in the heads of rootstock mother vines to negatively affect cane morphology, including thickness, mass and primary- and secondary lengths, while Celotheliaceae and Diaporthaceae decreased starch content in one-year-old canes.

Another strategy to combat the infection of one-year-old canes in rootstock mother blocks is to trellis rootstock mother vines. Training canes to grow horizontally, vertically, or at a 45° angle helps keep them off the ground (Waite et al., 2018). Effective trellis systems involve early-season shoot thinning and positioning canes on wires as they develop. This method is noted to produce larger quantities of high-quality straight cuttings compared to vines allowed to sprawl on the ground (Gramaje and Di Marco, 2015), but is highly labour-intensive (Hunter et al., 2004). Canes will subsequently not be in direct contact with the soil surface, which may serve as a source of inoculum for certain GTDs. This strategy also results in more consistent cane ripening, ensuring the full length of the cane can be used for grafting of new vines (Hunter et al., 2004). Additionally, the use of trellising facilitates the growth of a longer trunk, simplifying the process of trunk renewal in case of vine infection. Trunk renewal involves the removal of the infected wood and an additional 10 - 20 cm of non-symptomatic wood from infected vines and water shoots to replace the missing vine or cordon (Sosnowski et al., 2021). Regularly renewing the trunks of short-trunk and trellised vines is advantageous in minimising inoculum levels within the crowns of mother vines (Calzarano et al., 2004) and is suggested to be done on five to ten-year intervals, to reduce the likelihood of systemic infections and delay the need to replace mother vine plantings (Waite et al., 2018). However, trunk renewal needs to be accompanied by proper wound sealing and healing.

Attempts have been made in the past to link physiological and morphological parameters of graft material to the performance of grafted vines, without conclusive involvement of GTDs.

However, the adverse effects of GTDs can be observed in nurseries as grafting failure due to poor callus formation, reduction in the percentage of shoots emerging from dormancy, slow establishment following planting or death of graftlings. These pathogens, with specific emphasis on *Pa. chlamydospora* and *D. seriata*, establish in the graftlings and negatively affect callus and development thereof. A correlation between *D. seriata* DNA concentration in canes before grafting and incidence in the graft union after uprooting suggests that the inoculum of this pathogen establishes and increases during the nursery phase. *Phaeomoniella chlamydospora* was found to have a negative impact on root development in nursery vines. Furthermore, synergistic interactions were observed among different GTD pathogens, where infection by certain pathogens may predispose the graftlings to infection by other pathogens. The adverse effect of *Pa. chlamydospora*, together with other GTDs, on callus formation and grafting development in the propagation process may be reduced by the implementation of a soak in surface sterilant and HWT once the canes are harvested. Hot water treatments on dormant cuttings and young vines have been proven to suppress various GTD pathogens, such as *Pa. chlamydospora* and *Phaeoacremonium* spp. (Gramaje et al., 2009), Botryosphaeriaceae spp. (Billones-Baaijens et al., 2015a) and black foot pathogens (Halleen et al., 2007). While it doesn't completely eliminate all pathogens (Billones-Baaijens et al., 2015b; Elena et al., 2015; Eichmeier et al., 2018), combining HWT with fungicides may offer greater efficacy than either treatment alone (Serra et al., 2011).

Although resulting in higher root masses of graftlings during the nursery process, excessive nitrogen application may lead to reduced certifiable yields due to higher vine failure rate as well as more severe infections by certain GTD pathogens and, therefore, needs to be carefully applied. Excessive nitrogen fertilisation can result in overly vigorous growth in the mother blocks, which leads to dense vines with poor sunlight exposure of canes (overshadowing), poor cane ripening, poor nitrogen and carbohydrate reserve accumulation, higher levels of insect vectors and other pests, and canes that are thicker than the standards. The direct effect of minerals on the growth of GTD pathogens needs to be further investigated. For instance, this study has shown a negative correlation between Potassium and *Pa. chlamydospora*, magnesium and *Diaporthe*, and boron and *Pl. richardsiae*, while zinc correlated positively with Botryosphaeriaceae and *Diaporthe* incidences in grafted vines. In vitro experiments can aid in unravelling the effect of these minerals on the growth of the pathogens and may lead to individualized fertilizing regimes for rootstock mother blocks.

Vines grafted from older blocks had higher black foot pathogens and *Pl. richardsiae* incidences than those grafted from younger blocks. This was, however, not influenced by physiological and morphological status, nor by previous infections within one-year-old canes. Transcriptomics and metabolomics studies may shed light on the phenomenon observed in the current study. The nursery process has been implicated as the only source of infection of black foot disease and *Pl. richardsiae*, which forms part of the Petri disease complex. This can be ascribed to the use of the same nursery soils for decades in South Africa, where only a two-year rotation cycle is implemented, leading to a build-up of soil-borne inoculum. However, this is unavoidable and alternative control measures should, therefore, be implemented. Cartonnage, for instance, is a technique whereby grapevines are propagated in disease-free soils in compostable containers, and is already used at Vititec (PTY) LTD in South Africa. However, it should be considered to implement this technique on a larger scale in the South African grapevine industry, as it may result in lowered GTD infection, higher certifiable yield, and avoid root damage when the vines are planted in the field. This could be investigated in follow-up studies. With regards to this technique ,HWT should always be incorporated before the grafting of vines in order to reduce infection pressure originating from contaminated rootstock material.

For a nursery, the removal of a mother block has many practical and financial implications. Results from the current study suggest that the impacts of ageing rootstock mother blocks can be mitigated by proper management of rootstock mother blocks. This entails the use of pruning wound protectors and implementing sanitation practices in rootstock mother blocks to prevent infection by GTDs and consistently produce healthy canes. Furthermore, rootstock mother vines need to be adequately watered and correctly fertilised. With these strategies incorporated, and when a rootstock mother block produces canes with satisfactory phytosanitary status, sufficient cuttings, and with an above-average grafting percentage, rootstock mother blocks can be utilised for up to 10 years. Knowledge generated through this project provides local grapevine nurseries, rootstock mother block owners and the Plant Improvement Scheme with scientifically based information and guidelines on the management of the productive lifespan of rootstock mother blocks in South Africa.

9. PLANNED OUTPUTS

a) TECHNOLOGY DEVELOPMENT, PRODUCTS AND PATENTS

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

Final work will be presented at suitable conferences and local information days.

c) HUMAN RESOURCES DEVELOPMENT / TRAINING (STUDENTS)

Student Name and Surname	Student Nationality	Degree (e.g., Hons, MSc)	Level of studies in the final year of the project	Total Bursary Cost for Industry for the entire project
Honours				
Masters				
PhD				
Wynand van Jaarsveld	South African	PhD	Graduated	R 397150
Postdocs				

d) LIKELY PUBLICATIONS (POPULAR, PRESS RELEASES, SCIENTIFIC)

PhD dissertation
3x scientific publications
1x popular publication (Winetech Tegnies)

e) PRESENTATIONS/PAPERS THAT COULD BE DELIVERED

10th International Table Grape Symposium (Somerset-West, South Africa, 26 November – 1 December 2023)
Do grapevine trunk disease pathogens increase with rootstock mother vine age?
W.J. van Jaarsveld, L. Mostert, J.J. Hunter, M. Havenga & F. Halleen

International Congress of Plant Pathology (Lyon, France, 20-25 August 2023)
Grapevine trunk disease pathogens in rootstock mother vines: a potential threat to the South

African grapevine industry.
W.J. van Jaarsveld, L. Mostert , M. Havenga & F. Halleen

University of Stellenbosch (Dep. Plant Pathology, Friday Forum 5 July 2019).
Determining factors that may shorten the productive lifespan
of grapevine rootstock mother plantations: the proposal.
W.J. van Jaarsveld, L. Mostert, J.J. Hunter & F. Halleen

University of Stellenbosch (Dep. Plant Pathology, Friday Forum 14 February 2020).
Observations after one season's survey of canes and heads of grapevine rootstock mother blocks.
W.J. van Jaarsveld, L. Mostert, J.J. Hunter & F. Halleen

University of Stellenbosch (Dep. Plant Pathology, Friday Forum 5 November 2021).
Comparing the cane morphology and gtd infection levels of grapevine rootstock mother plants at different ages.
W.J. van Jaarsveld, L. Mostert, J.J. Hunter & F. Halleen

University of Stellenbosch (Dep. Plant Pathology, Friday Forum 14 October 2022).
THE EFFECT OF GRAPEVINE CANE HEALTH ON CALLUSING AND NURSERY VINE PERFORMANCE
W.J. van Jaarsveld, L. Mostert, J.J. Hunter & F. Halleen

South African Society for Plant Pathology Western Cape Branch symposium (Stellenbosch, South Africa, 3 April 2023).
Determining factors that may shorten the productive lifespan of grapevine rootstock mother plantations
W.J. van Jaarsveld, L. Mostert, J.J. Hunter & F. Halleen

Other presentations that could be delivered:
SASEV (conferences to be announced).

10. PROJECT OUTCOME AND IMPACT

New Knowledge	Benefits Supply Chain	Direct Grower Application	Direct Packhouse/Winery/ Cellar Application	Other
X		X		

Other is:

The Value of the project to the industry

Knowledge generated through this project will provide local grapevine nurseries, rootstock mother block owners and the Plant Improvement Scheme with scientifically based information and guidelines on the management of the productive lifespan of rootstock mother blocks in South Africa.

11. PERSONS PARTICIPATING IN THE PROJECT:

INITIALS AND SURNAME	HIGHEST QUALIFICATION	RACE (B, C, I, W)	GENDER (M, F, X)	INSTITUTE DEPARTMENT	POSITION	TOTAL COST TO PROJECT
RESEARCH PERSONNEL						R 0
Francois Halleen	PhD	W	M	ARC Infr-Nietv	PL	R 0
Lizel Mostert	PhD	W	F		Coll	R 0
Kobus Hunter	PhD	W	M	ARC Infr-Nietv	Co	R 0
Neels Volschenk	MSc	W	M	ARC Infr-Nietv	Co	R 0
Minette Havenga	PhD	W	F	ARC Infr-Nietv	Co	R 0
SUPPORT PERSONNEL						R 0
Palesa Lebenya	MSc	B	F	ARC Infr-Nietv	TA	R 0
Danie Marais	Matric	W	M	ARC Infr-Nietv	RA	R 0
Alwina Marais	Matric	W	F	ARC Infr-Nietv	RA	R 0
Julia Marais	Matric	W	F	ARC Infr-Nietv	RA	R 0
Carine Vermeulen	Matric	W	F	ARC Infr-Nietv	RA	R 0

POSITION: Co = Co-worker (other researcher at your institution)
Coll = Collaborator (participating researcher that does not receive funding for this project from industry)
PF = Post-doctoral fellow
PL = Project leader
RA = Research assistant
TA = Technical assistant/ technician

12. TOTAL COST OF PROJECT

TOTAL ANNUAL COSTS (ALL YEARS)	CFPA	RAISINS SA	HORTGRO	SATI	WINETECH	ARC	OTHER	TOTAL
2018 and before	R 0	R 0	R 0	R 0	R 0	R 0	R 0	R 0
2019	R 0	R 0	R 0	R 0	R 335083	R 255087	R 0	R 590170

2020	R 0	R 0	R 0	R 0	R 379113	R 294669	R 0	R 673782
2021	R 0	R 0	R 0	R 0	R 404633	R 315296	R 0	R 719929
2022	R 0	R 0	R 0	R 0	R 467852	R 374015	R 0	R 841867
2023	R 0	R 0	R 0	R 0	R 0	R 0	R 0	R 0
TOTAL	R 0	R 0	R 0	R 0	R 1586681	R 1239067	R 0	R 2825748