Research Article

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Adverse effects of grapevine trunk diseases on canes produced from infected rootstock mother blocks

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Abstract

BACKGROUND: Grapevine trunk diseases (GTDs) threaten vineyard longevity by reducing the yield and phytosanitary quality of propagation material from rootstock mother vines. The optimal age for replacing mother blocks to mitigate these risks is unclear. This study evaluated the effects of mother block age on the physiological, morphological, and infection status of mother vines and one-year-old canes produced by it.

RESULTS: Cane morphology and physiology were highly variable, influenced by rootstock and season, with no consistent association with mother block age. Grapevine trunk disease incidence was widespread in mother vine heads, dominated by Celotheliaceae and Botryosphaeriaceae, with incidences up to 84-90%. One-year-old canes generally showed lower infection rates, though Botryosphaeriaceae and Celotheliaceae reached 28% and 2%, with DNA concentrations up to 3171 and 1055 ng μL^{-1}, respectively. *Diplodia seriata* in mother vine heads directly contaminated one-year-old canes. Multivariate analyses revealed Botryosphaeriaceae negatively impacted cane morphology, Celotheliaceae and Diaporthaceae reduced starch content, and Botryosphaeriaceae and Hymenochaetaceae increased total phenolic index.

CONCLUSION: Mother block age minimally influenced cane physiology or morphology. However, high GTD prevalence in mother vine heads poses systemic infection risks and reduces cane quality. Monitoring and management of GTD pathogens in rootstock mother blocks are essential to maintain the health and productivity of propagation material and ensure vineyard longevity.

Supporting information may be found in the online version of this article.

Keywords: productive lifespan; cane morphology; vine physiology; *Phaeomoniella chlamydospora*; *Diplodia seriata*

1 INTRODUCTION

The utilization of rootstocks has played a pivotal role in the South African wine industry's success, enabling growers to overcome challenges posed by grapevine pests and diseases. Currently, most grapevines in South Africa are grafted onto meticulously chosen rootstocks that are selected based on their desired attributes. A 10-year rotation plan is implemented by some rootstock mother block owners in South Africa, while others use rootstock mother blocks for as long as it remains economically viable. The risks of aging rootstock mother vines becoming infected with GTDs such as Petri disease, Esca, Botryosphaeria dieback, Phomopsis dieback and Eutypa dieback, and growers supplying propagation material with compromised phytosanitary status could seriously impact the longevity of grapevines and the economic viability of the industry. For instance, Nader *et al.*[1] assessed the influence of vine age on various physiological and reproductive parameters and identified GTDs as the primary factor contributing to vine decline. Although the Vine Improvement Association in South Africa makes provision for several requirements (i.e., production units, virus and viroid status, physical dimensions),[2] none of these address the productive lifespan of rootstock mother blocks.

Grapevine trunk diseases primarily spread through airborne spores[3] originating from hyphomycete structures, pycnidia or perithecia on colonized grapevine wood[4–7] that are associated

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with sufficient periods of precipitation.[4,8–10] The inoculum spreads through wind, water splashing, arthropods and pruning tools.[5,8,11–13] Grapevine rootstock mother plants are particularly susceptible to infection through aerial inoculum since all the canes of rootstock mother plants in South Africa are pruned at the base. The open wounds directly expose the perennially retained trunk to infection, unlike grapevine source plants used for scion cuttings.[3,11,14–19] Consequently, rootstock mother vines accumulate GTDs over time which eventually lead to decay and vine head deterioration.[3,11,20–22]

Multiple research studies have consistently demonstrated that canes from infected rootstock mother vines are the primary source of GTD inoculum.[18,23–43] Pathogens residing within the rootstock mother vines' woody parts can infect one-year-old rootstock canes through various mechanisms including contamination by conidia spreading through the vascular system. This is facilitated by the flow of plant sap,[11,31,44–47] infection through the active growth of hyphae within canes after budburst[17,31] and epiphytic inoculum that directly penetrates the bark of canes and results in infection.[19,48]

The primary physiological impact of GTDs revolves around the regulation of carbohydrate metabolism and the activation of defence mechanisms in various parts of the grapevine.[49] According to Fontaine *et al.*,[49] these effects manifest in distinct ways within different parts of the vine. In the trunk, GTDs lead to a reduction in starch reserves within woody tissues, often coinciding with fungal infection.[49] Meanwhile, in the leaves, where these pathogens do not reside, carbohydrate metabolism is affected as indicated by a decline in the photosynthetic rate.[50] Canes from symptomatic plants tend to have reduced carbohydrate reserves during the winter period.[51] Carbohydrates stored in perennial organs play a vital role in supporting the early development of annual plant parts during spring.[52] Consequently, disruptions in carbohydrate reserves can contribute to a decline in the plant's growth and vitality in the following growth season.[49,51] The early phase of the plant's defence response to infection includes the formation of polyphenol-rich reaction zones at the infection site, i.e., the build-up of phenolic compounds in the xylem vessels, effectively restricting the growth and dissemination of fungal hyphae.[49,53–55] Various grapevine tissue types exhibit variability in phenolic build-up, with the highest accumulation observed in young shoots in comparison to the trunk.[55] Studies investigating the physiological status of one-year-old canes in relation to vine age and GTD infection status are lacking.

This study aimed to elucidate the effect of rootstock mother block age on fungal infection as well as morphological and physiological parameters of one-year-old canes that may influence nursery vine performance. The objectives were to determine (i) morphological dimensions of one-year-old canes including primary and secondary cane lengths, cane thickness and cane mass, (ii) the physiological status of one-year-old canes including starch content, total mineral content, total phenolic index (TPI) and water content, (iii) the incidence of GTD pathogens in one-year-old canes and heads of mother vines and (iv) the relationship between grapevine physiology, morphology and GTD pathogen incidence of both the canes and heads in rootstock mother blocks.

2 MATERIALS AND METHODS

Mother vine heads and one-year-old canes in blocks of different ages of the most widely propagated grapevine rootstock varieties in South Africa were surveyed over three seasons (2019, 2020, 2021). Varieties included Millardet et de Grasset 143 B, Millardet et de Grasset 101–14, Paulsen 1103, Ramsey, Richter 110, Richter 99, Ruggeri 140, Selektion Oppenheim 4 and US VIT 8–7 (Supporting Information, Table S1). All experimental plots were located in the Wellington region of the Western Cape Province, South Africa (Köppen-Geiger climate classification Csa – temperate humid with cool winter and summer drought and mean temperature of the warmest month >22 °C), within a 16.5 km range. Standard cultural practices, including cane sprawling and weekly irrigation, were applied in all experimental plots during the growing season. In each experimental plot, five well-spaced zones were randomly selected across the vineyard, and within each zone, 10 plants in a row were labelled.

2.1 Sampling of one-year-old canes

One dormant cane that met the plant propagation standards in South Africa was sampled from each labelled plant in all the experimental plots during the same time in autumn of each season and transported to the laboratory, and stored at 4 °C until analysis.

2.1.1 *Morphological dimensions*

The primary and secondary cane lengths of each cane were measured. The dorsal and lateral thickness of each cane was measured at the 4th internode from the basal end using a digital caliper. The fresh mass of all canes originating from each zone was weighed.

2.1.2 *Physiological analysis*

2.1.2.1. Water content. Water content was determined by pooling the third internode of all canes per zone, resulting in five pooled samples per block. After weighing the fresh cane internode mass of each zone, samples were lyophilized for 168 h and the dry cane mass weighed. The equation used for the determination of water content was:

$$\text{Water content} = \frac{\text{Fresh mass (g)}}{\text{Dry mass (g)}} \times 100$$

2.1.2.2. Mineral content. Mineral content was determined by grinding the lyophilized cane internode samples to chips using a standard model no 3 Wiley mill (Arthur H. Thomas Co., Philadelphia, U.S.A) followed by grinding it to a fine powder in a Cyclotec sample mill (Tecator, Sweden) at room temperature. The samples were analyzed by a SANAS accredited (ISO 17025:2017) testing laboratory, KL Analytical Services CC T/A Labserve (T0467). The total nitrogen concentration of the samples was determined[56] using the wet oxidation digestion procedure.[57] The other macro- and micronutrients (P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn, B, S and Mo) were analyzed using wet digestion with nitric-perchloric acid.[58]

2.1.2.3. Starch concentration. The starch concentration of plant tissue was determined from the lyophilized ground cane samples using a method described by Hunter *et al.*[59] and adapted by Theron.[60]

2.1.2.4. Total phenolic index. Total phenolic index was determined using an adapted method of Ribéreau-Gayon *et al.*[61] A 1 g lyophilized ground cane sample was mixed with 10 mL buffer solution [5 g tartaric acid, 22 mL 1 N sodium hydroxide (NaOH),

2 g sodium metabisulfite ($Na_2S_2O_5$) and 120 mL absolute ethanol made up with deionized water to 1 L and pH adjusted to 3.2 using 1 M NaOH] and incubated at 30 °C for 7 days. Samples were shaken every day for 10 s. Following the incubation period, 2 mL of the buffer/sample mix was transferred to a 2 mL microcentrifuge tube and centrifuged at 12 000 rpm for 10 min. The supernatant was diluted with double distilled water (ddH_2O) as necessary. Absorbance was read at 280 nm using a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, South Africa). The equation used for the determination of TPI was:

$$\text{TPI} = \text{Abs280} \times \text{dilution factor}$$

2.1.3 *Fungal incidence of one-year-old canes in 2019 season*

The first basal internode of each one-year-old cane from the 2019 season was split longitudinally, surface sterilized (successively 30 s in 70% ethanol, 1 min in 3% sodium hypochlorite, and 30 s in 70% ethanol) and air-dried in a laminar flow cabinet. Twelve sections (2 × 1 mm) were cut from both the xylem and pith and placed on three potato dextrose agar (PDA, Biolab, South Africa) Petri dishes amended with chloramphenicol (PDA—C; 250 mg L^{-1}). The Petri dishes were sealed with Parafilm® and incubated at 25 °C under a 12-h fluorescent white light/dark regime and inspected for new fungal growth daily.

2.2 Sampling of mother vine heads

Heads of the same rootstock mother vines that were used to determine the morphological and physiological characteristics and fungal incidence of one-year-old canes were sampled. One wood cross-section ranging between 30 - 90 mm was cut from the side of each mother vine head using a reciprocating saw with a sterilized cutting blade in late winter of each season, transported to the laboratory and stored at 4 °C until analysis.

2.2.1 *Fungal incidence in heads of rootstock mother vines in 2019 and 2020 seasons*

Symptoms in each cross section were photographed. Sections containing soft rot were removed and flame sterilized by holding it with sterile forceps, lightly spraying it with 70% ethanol and passing it through a flame. The remainder of the cross-section was surface sterilized as described above. Subsequently, four wood pieces (2 × 1 mm) were removed from each occurring symptom and transferred to one PDA-C Petri dish. The Petri dishes were handled as described above.

2.3 Identification of fungal isolates

2.3.1 *Morphological identification of fungal isolates*

The fungal isolates were grouped into families of Botryosphaeriaceae, Celotheliaceae, Diaporthaceae, Diatrypaceae, Hymenochaetaceae and Togniniaceae based on cultural growth characteristics. *Phaeomoniella chlamydospora* colonies were identified morphologically.[62] The Botryosphaeriaceae isolates were plated out on water agar (WA, Biolab, South Africa) with double-autoclaved pine needles and incubated at 24 °C under a 12-h near-ultraviolet light/dark regime for 2–4 weeks, or until conidiomata formed. Microscope slides were prepared for each isolate and subsequently grouped into genera based on conidia morphology at 400× magnification.[63–65]

2.3.2 *Molecular identification of fungal isolates*

Genomic DNA was extracted from fungal mycelium as described by Havenga *et al.*[66] Various species-specific polymerase chain reactions (PCRs) were first conducted using Ampliqon Taq DNA Polymerase Master Mix RED (Biomol, Germany) to identify isolates up to species level. The PCR products were separated by electrophoresis alongside a 100-bp DNA ladder (GeneRuler™, Thermo Fisher Scientific, Waltham, Massachusetts, USA) on a 1% (w/v) agarose gel in TAE running buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.5) stained with SYBR™ Safe DNA Gel Stain (Invitrogen, Thermo Fisher Scientific, Carlsbad, USA). The GeneGenius Gel Documentation and Analysis System (Syngene, UK) was used to visualize the gel under ultraviolet light.

For putative Togniniaceae isolates, species-specific PCRs were conducted to identify *Phaeoacremonium parasiticum* (Pbr2_2, T1) and subsequently *P. minimum* (Pbr6_1, T1).[9] For putative Diaporthales isolates, PCRs were performed to detect *Diaporthe foeniculina* (Dfoen_F1, Dfoen_R1), followed by *D. ampelina* (Damp_F1, Damp_R2) and *D. ambigua* (Dambigua_f1, Dambigua_R2).[67] Putative Botryosphaeriaceae isolates lacking conidiomata, as well as representative isolates of *Diplodia seriata* identified morphologically, were screened with *D. seriata*-specific primers DS3.8S3 and DS3.8R4.[68] All putative *Neofusicoccum* isolates were subjected to *Neofusicoccum parvum* primers Bot_17 and Bot_18.[69,70] For Hymenochaetales, PCRs were conducted with *Fomitiporia capensis* primers BT9 and ITS4,[71,72] followed by Taxon 1 (*Fomitiporella* sp.) primers BT1 and ITS4. Putative Diatrypaceae isolates were screened using *Eutypa lata* primers EL1 and EL4[73] and subsequently *Cryptovalsa ampelina* primers Camp1 and Camp2R.[74]

Housekeeping genes were amplified and sequenced for isolates that failed species-specific PCR amplification, as well as for representative isolates confirmed by species-specific PCRs. PCR products were purified with the PreIT ExoZap PCR clean-up kit (Ampliqon A/S, Denmark) and sequenced at the Central Analytical Facility (CAF), Stellenbosch University, South Africa. For Togniniaceae, the partial beta-tubulin gene (TUB) was amplified using primers T1[75] and Bt2b.[76] For Diaporthales, Hymenochaetales, and Diatrypaceae, the ribosomal RNA Internal Transcribed Spacer (ITS) region was amplified using primers ITS5 and ITS4.[72] For Botryosphaeriaceae, the elongation factor 1-alpha (EF-1α) gene was amplified using primers EF688F and EF1251R.[77]

The DNA sequences were trimmed and edited in Geneious® 11.1.5 (Biomatters Ltd., Auckland, New Zealand). Preliminary identifications were obtained with BLASTn (Basic Local Alignment Search Tool for nucleotide) searches against the NCBI nucleotide databases (http://www.ncbi.nlm.nih.gov/Genbank). Reference sequence data for each taxonomic group were obtained from NCBI database. Sequence data sets for each taxonomic group were aligned with the L-INS-I algorithm using the program MAFFT 7.450[78] in Geneious®. Maximum likelihood (ML) analyses were done using PHyML 2.2.4[79] under the general time reversible (GTR) model. The proportions of invariable sites and gamma distribution parameters were estimated. Bootstrap support values were calculated from 100 replicates. Clades with bootstrap support of ≥70% were considered significant.[80] The resulting trees were plotted using FigTree 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree).

2.4 Quantitative real-time polymerase chain (qPCR) reactions of one-year-old canes over three seasons

2.4.1 *Genomic DNA extraction and standards preparation*

Genomic DNA was extracted from the lyophilized, ground cane samples of three seasons (2019, 2020, 2021) using the Qiagen

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DNeasy® Plant Mini Kit (Qiagen GmbH, Qiagen Strasse 1, 40 724 Hilden, Germany) and used for DNA quantification of *Pa. chlamydospora*[81] and *D. seriata*.[82] Serial five-fold dilutions from 25–12.8 fg μL^{-1} of both *Pa. chlamydospora* and *D. seriata* DNA templates were used to determine the level of detection (LOD) and level of quantification (LOQ) within acceptable qPCR parameters.[83] The concentration standards were carried out in triplicate and the cane DNA samples were carried out in duplicate.

2.4.2 *Inhibition testing*

The DNA of a cane sample with no *Pa. chlamydospora* infection was selected to test for qPCR reaction inhibitors. Inhibitors could originate from the cane samples or co-purified compounds during the DNA extraction. Undiluted and 3-, 5-, 10-, 20-, 30-, 40- and 50-fold diluted DNA samples were used. All samples were spiked with *Pa. chlamydospora* DNA to a final concentration of 10 ng μL^{-1}. For the positive control, nuclease free water was spiked with *Pa. chlamydospora* DNA to the same concentration. No-template control samples received nuclease-free water. The qPCR reactions were done according to the assay described above. Reaction inhibition was assessed by comparing Cq values of the diluted samples with those of the positive control.

2.5 Statistical analyses

2.5.1 *One-year-old canes morphological and physiological analyses*

For each mother block, per cane assessed data were summarized on a zone level. Zones represented five random replicates for a specific mother block. Analysis of variance (ANOVA) was conducted on variables assessed to compare mother block ages only for each season and clone separately. Shapiro–Wilk test was performed on the standardized residuals from the model to test for deviation from normality.[84] Fisher's least significant difference was calculated at the 5% level to compare treatment means for significant effects. A probability level of 5% was considered significant for all significance tests. Univariate analyses were done using SAS statistical software (Version 9.4, SAS Institute Inc., Cary, NC, USA).

2.5.2 *Disease incidence of one-year-old canes and heads of rootstock mother blocks*

Each mother block's survey data was summarized at zone level. Zones represented five random replicates for a specific mother block. The percentage incidence of each pathogen was calculated for each zone of each mother block in each season as the percentage of plants for which a specific pathogen was isolated out of the total number of plants observed. The data were then subjected to ANOVA using General Linear Models Procedure (PROC GLM) of SAS software to compare mother block ages only for each season and clone separately. Shapiro–Wilk test was performed on the standardized residuals from the model to test for deviation from normality.[84] Fisher's least significant difference was calculated at the 5% level to compare treatment means for significant effects. A probability level of 5% was considered significant for all significance tests. Univariate analyses were done using SAS statistical software.

2.5.3 *Quantitative real-time polymerase chain reaction of one-year-old canes over three seasons*

The averages of absolute amounts of *D. seriata* and *Pa. chlamydospora* DNA determined by qPCR in two independent technical replicates were standardized on the amounts of input DNA and used for further statistical analysis. The data were then subjected to ANOVA using General Linear Models Procedure (PROC GLM) of SAS software. Shapiro–Wilk test was performed on the standardized residuals from the model to verify normality.[84] The DNA concentrations deviated from normality and were Ln(X + 1) transformed to improve normality. Fisher's least significant difference was calculated at the 5% level to compare treatment means for significant effects.[85] A probability level of 5% was considered significant for all significance tests. Univariate analyses were done using SAS statistical software.

2.5.4 *Multivariate analyses*

Pearson correlations were done between *D. seriata* and *Pa. chlamydospora* percentage incidence in canes and heads, and DNA concentrations for the 2019 season. Principal Component Analysis (PCA) that included the same data was done to elucidate associations of mother block cultivars and ages with these variables. The morphological dimensions (primary and secondary cane lengths, fresh cane mass) and physiological analysis (water content, mineral analysis, starch content, TPI) conducted in the 2019 and 2020 seasons on the same canes as used for qPCR, and from the same vines than used for head isolation, were included in multiple factor analysis in order to observe associations amongst cane- and mother vine health status. Multivariate analyses were conducted using XLStat [Addinsoft (2022), XLSTAT statistical and data analysis solution. New York, USA. https://www.xlstat.com/en].

3 RESULTS

3.1 Morphological dimensions

Cane morphology differences between younger and older rootstock mother blocks were highly variable and largely rootstock- and season-specific (Supporting Information, Table S2, multivariate analysis). For primary cane length, younger blocks of Paulsen 1103 PS28I, Richter 99 RY13C, SO4 SO332A, and US 8–7 UC274A often produced longer canes, while Ruggeri 140 RU354E showed the opposite trend. Secondary cane length patterns were inconsistent, with older blocks of 143 B BA31C and Richter 99 RY13C yielding longer canes in some seasons, whereas younger blocks of Ruggeri 140 RU354E, SO4 SO332A, and US 8–7 UC274 produced longer canes in others. Cane mass trends were similarly variable, and cane thickness showed the least consistent differences. Overall, no uniform trends were observed across rootstocks or seasons.

3.2 Physiological analyses

Physiological traits of one-year-old canes from rootstock mother blocks of different ages showed highly variable, rootstock- and season-specific trends (Supporting Information, Table S3, multivariate analysis). Starch content was generally higher in younger blocks of 143 B BA31C and Ramsey SC18AB, but older blocks of Paulsen 1103 PS28I, Ramsey SC18AE, and SO4 SO332A often had higher starch levels. The TPI showed inconsistent patterns, with older blocks of 143 B BA31C sometimes higher, while SO4 SO332A had consistently higher TPI in younger blocks. Water content varied seasonally and by rootstock, with both older and younger blocks alternately recording the lowest or highest values. Mineral content showed minimal age-related trends. Overall, no consistent physiological patterns were observed across rootstocks or seasons.

3.3 Disease incidence in one-year-old canes

Trunk disease incidence in one-year-old cuttings from grapevine rootstock mother blocks varied by rootstock and planting year, with Botryosphaeriaceae most prevalent (Table 1). Older blocks of 143 B BA31C (32% in 2009), Paulsen 1103 PS28I (24% in 2009), and US 8–7 UC274A (26–28% in 2006–2008) showed the highest incidence, while younger plantings of 143B BA31C (0–8% in 2014–2018), Paulsen PS28I (0–2% in 2014–2018) and US8-7 UC274A (2–14% in 2012–2016) had much lower incidences. Other rootstocks, including Ramsey, Richter 99 RY13C, and SO4 SO332A, showed consistently low or variable incidence.

3.4 Disease incidence in mother vine heads

Higher incidences of GTDs have been recorded in older blocks, albeit still high in younger blocks (Table 2). For instance, for 143B BA31C, incidences of 78–100% were recorded in blocks from 2006 to 2009, compared to 70% in the 2014 block during the 2020 season. A similar trend was observed in Paulsen PS28I, where significantly higher incidences of GTDs were recorded in the 2009 and 2011 blocks compared to the 2014 and 2016 blocks over both seasons. The same pattern was seen in Ramsey SC18AB, with significantly higher incidences in the 2008–2011 blocks (85.56–98%) compared to the 2014 block (34–66%) across both seasons. This trend was also observed in Ramsey SC18AE, with incidences of 76–94% in the 1999–2012 blocks, which differed significantly

Table 1. Incidences of pathogens in one-year-old canes from different aged rootstock mother blocks in the 2019 season

Rootstock variety	Plant year	Total incidence*	Botryosphaeriaceae	Celotheliaceae	Diaporthaceae	Pleurostomataceae	Togniniaceae
143 B BA31C	**2006**	18.00ab†	18.00	0.00	0.00	0.00	0.00
	2008	16.00ab	16.00	0.00	0.00	0.00	0.00
	2009	32.00a	28.00	0.00	4.00	0.00	0.00
	2014	8.00b	8.00	0.00	0.00	0.00	0.00
	2018	0.00b	0.00	0.00	0.00	0.00	0.00
	LSD‡	19.479	19.027	.	3.2316	.	.
	***P*-value**	0.0318	0.0606	.	0.0623	.	.
Paulsen 1103 PS28I	**2008**	6.00b	6.00b	0.00	0.00	0.00	0.00
	2009	24.00a	22.00a	2.00	0.00	0.00	0.00
	2011	4.00b	4.00b	0.00	0.00	0.00	0.00
	2014	2.00b	2.00b	0.00	0.00	0.00	0.00
	2016	2.00b	2.00b	0.00	0.00	0.00	0.00
	2018	0.00b	0.00b	0.00	0.00	0.00	0.00
	LSD‡	11.05	11.553	3.5703	.	.	.
	***P*-value**	0.0016	0.0071	0.4389	.	.	.
Ramsey SC18 AB	**2008**	4.00	4.00	0.00	0.00	0.00	0.00
	2010	6.00	2.00	0.00	4.00	0.00	0.00
	2011	4.00	4.00	0.00	0.00	0.00	0.00
	2014	2.00	2.00	0.00	0.00	0.00	0.00
	2016	6.00	6.00	0.00	0.00	0.00	0.00
	LSD‡	10.879	9.8726	.	5.2771	.	.
	***P*-value**	0.9321	0.9063	.	0.4307	.	.
Ramsey SC18AE	**1999**	0.00	0.00	0.00a	0.00a	0.00	0.00
	2003	5.00	3.00	1.00	1.00	0.00	0.00
	2007	0.00	0.00	0.00	0.00	0.00	0.00
	2012	4.00	4.00	0.00	0.00	0.00	0.00
	2014	10.00	10.00	0.00	0.00	0.00	0.00
	2017	0.00	0.00	0.00	0.00	0.00	0.00
	LSD‡	8.5121	7.7307	2.1817	2.1817	.	.
	***P*-value**	0.1500	0.1127	0.8024	0.8024	.	.
Richter 110 RQ28C	**2008**	4.00	4.00	0.00	0.00	0.00	0.00
	2010	8.00	2.00	0.00	6.00	0.00	0.00
	2011	18.00	16.00	2.00	0.00	0.00	0.00
	2012	10.00	10.00	0.00	0.00	0.00	0.00
	2014	2.00	2.00	0.00	0.00	0.00	0.00
	LSD‡	13.71	11.8	2.6386	7.9157	.	.
	***P*-value**	0.1692	0.0917	0.4307	0.4307	.	.
Richter 99 RY13C	**2009**	2.00	2.00	0.00	0.00	0.00	0.00
	2012	2.00	2.00	0.00	0.00	0.00	0.00
	2014	4.00	4.00	0.00	0.00	0.00	0.00
	LSD‡	8.7153	8.7153	.	.	.	.

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Table 1. Continued

Rootstock variety	Plant year	Total incidence*	Botryosphaeriaceae	Celotheliaceae	Diaporthaceae	Pleurostomataceae	Togniniaceae
	***P*-value**	0.8484	0.8484	.	.	.	.
Ruggeri 140 RU354E	**2010**	10.00	10.00	0.00	0.00	0.00	0.00
	2014	12.00	6.00	0.00	2.00	2.00	2.00
	LSD‡	15.296	13.836	.	4.612	4.612	4.612
	***P*-value**	0.7707	0.5237	.	0.3466	0.3466	0.3466
SO4 SO332A	**2011**	8.00	4.00	0.00	4.00	0.00	0.00
	2012	12.00	10.00	0.00	2.00	0.00	0.00
	LSD‡	15.976	13.836	.	7.2922	.	.
	***P*-value**	0.5796	0.3466	.	0.5447	.	.
US 8–7 UC274A	**2006**	26.00a	22.00a	0.00	4.00	0.00	0.00
	2008	28.00a	26.00a	0.00	2.00	0.00	0.00
	2012	2.00b	2.00b	0.00	0.00	0.00	0.00
	2014	4.00b	2.00b	0.00	2.00	0.00	0.00
	2016	14.00ab	12.00ab	0.00	0.00	0.00	2.00
	LSD‡	19.833	15.721	.	6.4631	.	2.6386
	***P*-value**	0.0344	0.0109	.	0.6783	.	0.4307

*Percentage incidence of fungal families in one-year-old canes following isolations from 50 canes per block.
† Values followed by the same letter do not differ significantly within a column of a cultivar.
‡ Least significant differences ($P = 0.05$) and *P*-values of each column per cultivar indicated in the two bottom rows.

from the 2014 block (46–66%), over both seasons. In the 2019 season, a similar pattern was observed for Ruggeri 140 RU354E. For US8-7 UC274A, the lowest incidences were recorded in the 2016 block, which differed significantly from the 2006–2014 blocks in 2019 (52% *vs*. 75.56–96%) and from the 2006–2012 blocks in 2020 (72% *vs*. 89.56–100%).

Phylogenetic analysis revealed a large species diversity, with 14 Botryosphaeriaceae species, five Diaporthaceae species, three Diatrypaceae species, three Hymenochaetaceae species, three Togniniaceae species and one Celotheliaceae species (Supporting Information, Fig. S1–S9). *Phaeomoniella chlamydospora* was dominant with 9184 isolates followed by *D. seriata* (2353 isolates), *F. capensis* (2286 isolates), *P. minimum* (1184 isolates) and *D. foeniculina* (416 isolates) (Supporting Information, Table S4).

3.5 Internal symptoms in mother vine heads

A total of 68 936 isolations were made, and 21 405 fungal isolates were obtained from five symptom types as described by White *et al.*[86] (Table 3; Fig. 1). Celotheliaceae, Botryosphaeriaceae and Hymenochaetaceae were by far the most frequently isolated pathogens associated with these symptoms and represented 42.9%, 22.1% and 19.2% of the total isolates obtained, respectively. Most of the isolates were retrieved from the sectorial or undefined necrosis (8209 isolates) and black and brown wood streaking (7129 isolates), followed by necrosis in or adjacent to white rot (2538 isolates), white rot, usually central (1974 isolates), and black and brown margins adjacent to necrotic wood (1263 isolates).

3.6 qPCR of one-year-old canes

3.6.1 Standard curves

The standard curve for the *D. seriata* assay produced acceptable linear amplification with a linear coefficient (R^2) of 0.99574 and a reaction efficiency (E) of 0.99 for 10 consecutive standards. The linear regression equation produced from the standard curve, $Cq\ value = -3.351 \times \log(DNA\ concentration) + 18.686$, was used to calculate the initial DNA concentration of the canes in each zone. The LOQ and LOD as determined from the standard curve were 12.8 fg μL^{-1}. Substitution of the LOQ concentration (12.8 fg μL^{-1}) into the above equation, as determined from the standard curve, yielded a Cq threshold cut-off for non-specific detection of 34.68.

The standard curve for the *Pa. chlamydospora* assay produced acceptable R^2 of 0.99630 at an E of 0.94 for 10 consecutive standards. The linear regression equation produced from the standard curve, $Cq\ value = -3.471 \times \log(DNA\ concentration) + 16.151$, was used to calculate the initial DNA concentration of the canes in each zone. The LOQ and LOD as determined from the standard curve were 12.8 fg μL^{-1}. Substitution of the LOQ concentration (12.8 fg μL^{-1}) into the above equation, as determined from the standard curve, yielded a Cq threshold cut-off for non-specific detection of 32.70.

3.6.2 qPCR inhibition testing

The qPCR inhibition reactions indicated that no reaction inhibitors were present in undiluted samples. Detection was observed for the undiluted DNA extracted from wood samples and yielded a Cq of 14.27, similar to the 3-, 5-, 10-, 20-, 30-, 40- and 50-fold diluted background samples.

3.6.3 DNA quantification

Diplodia seriata and *Pa. chlamydospora* showed variable infection across rootstock varieties and planting years. *Diplodia seriata* was sporadic, with concentrations ranging from undetectable to 7.28 ng μL^{-1}, while *Pa. chlamydospora* persisted at higher levels (often >5 ng μL^{-1}) across most samples. Infection patterns were influenced by both rootstock and plant year, with significant

Table 2. Incidences of pathogens in rootstock mother vine heads of differently aged rootstock blocks

		Total incidence*		Botryosphaeriaceae		Celotheliaceae		Diaporthaceae		Diatrypaceae		Hymenochaetaceae		Togniniaceae	
Rootstock variety	Plant year	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
143 B BA31C	**2006**	88.00	100.00a†	50.00	67.11	54.00ab	87.78a	0.00	14.44	0.00	6.00	10.00	71.33a	16.00	78.00a
	2008	90.00	78.00bc	80.00	56.00	36.00b	50.00b	4.00	2.00	0.00	0.00	2.00	4.00b	4.00	8.00b
	2009	94.00	96.00ab	70.00	50.00	64.00a	76.67a	12.00	12.67	8.00	2.22	4.00	2.22b	6.00	6.00b
	2014	80.00	70.00c	59.97	52.00	8.75c	14.00c	7.738	16.00	0.00	0.00	0.00	4.00b	6.67	8.00b
	LSD‡	28.702	19.545	35.989	26.875	24.802	24.134	9.093	14.478	7.8604	6.8592	8.7882	8.4862	13.231	21.619
	***P*-value**	0.7872	0.0140	0.3335	0.5509	0.0019	<0.0001	0.0612	0.2031	0.1031	0.2450	0.1313	<0.0001	0.2390	<0.0001
Paulsen 1103 PS28I	**2009**	98.00a	96.00a	58.00a	46.00	74.00a	82.00a	8.00	16.00	2.00	2.00	12.00b	20.00ab	24.00b	18.00
	2011	98.00a	94.00a	50.00a	28.00	88.00a	84.00a	6.00	8.00	0.00	2.00	32.00a	32.00a	48.00a	20.00
	2014	64.00b	70.00b	18.00b	28.00	44.00b	54.00b	6.00	8.00	0.00	0.00	0.00b	8.00b	4.00c	8.00
	2016	62.00b	70.00b	42.00a	44.00	12.00c	30.00c	16.00	24.00	2.00	0.00	8.00b	4.00b	2.00c	12.00
	LSD‡	14.839	14.378	22.335	21.723	14.99	21.515	14.378	14.221	4.2398	4.2398	17.222	16.005	14.99	19.079
	***P*-value**	<0.0001	0.0010	0.0094	0.1792	<0.0001	0.0002	0.4244	0.0875	0.5847	0.5847	0.0080	0.0080	<0.0001	0.5387
Ramsey SC18AB	**2008**	94.00a	92.00a	38.00a	40.00	82.00ab	78.00a	14.00	26.00	2.00	0.00	10.00b	12.00	16.00b	26.00a
	2010	94.00a	96.00a	26.00ab	28.00	68.00b	82.00a	16.00	32.00	0.00	2.00	58.00a	8.00	6.00b	6.00b
	2011	98.00a	85.56a	38.00a	34.89	90.00a	79.33a	12.00	12.44	0.00	0.00	28.00b	16.00	32.00a	12.00ab
	2014	34.00b	66.00b	6.00b	18.00	16.00c	44.00b	10.00	24.00	0.00	2.00	0.00c	8.00	4.00b	0.00b
	LSD‡	11.803	15.252	20.662	19.969	20.879	19.987	18.112	16.555	2.998	4.2398	23.125	18.236	15.139	17.481
	***P*-value**	<0.0001	0.0035	0.0143	0.1486	<0.0001	0.0027	0.9066	0.1282	0.4182	0.5847	0.0004	0.7574	0.0046	0.0354
Ramsey SC18AE	**1999**	76.00a	87.778a	42.00	24.67	52.00a	69.56a	6.00	18.44ab	0.00	0.00	10.00	20.22	16.00	30.67a
	2003	88.00a	89.00a	42.00	26.00	64.00a	75.00a	6.00	10.00bc	6.00	4.00	8.00	17.00	18.00	30.00a
	2007	80.00a	94.00a	42.00	42.00	60.00a	62.00a	4.00	6.00c	0.00	4.00	8.00	24.00	28.00	28.00ab
	2012	84.00a	94.00a	12.00	18.00	70.00a	78.00a	12.00	10.00bc	2.00	4.00	6.00	12.00	20.00	46.00a
	2014	46.00b	66.00b	28.00	30.00	18.00b	22.00b	10.00	20.00a	2.00	8.00	8.00	14.00	2.00	8.00b
	LSD‡	18.362	12.423	24.063	21.501	22.919	16.852	10.942	9.9254	6.9903	9.1142	10.009	15.452	17.194	20.213
	***P*-value**	0.0006	0.0008	0.0642	0.2912	0.0010	<0.0001	0.5846	0.0396	0.2446	0.5766	0.9601	0.5736	0.0856	0.0227
Richter 110 RQ28C	**2008**	100.00	95.78	68.00a	52.67	80.00a	77.78a	4.00b	4.22c	0.00a	4.00ab	60.00a	45.56a	46.00a	42.44a
	2010	80.00	96.00	40.00b	32.00	46.00b	56.00ab	0.00b	46.00a	0.00a	0.00b	30.00b	10.00b	12.00b	10.00b
	2011	96.00	96.00	56.00ab	58.00	66.00ab	68.00ab	0.00b	4.00c	0.00a	0.00b	74.00a	64.00a	10.00b	12.00b
	2012	94.00	92.00	64.00a	50.00	52.00b	46.00b	12.00ab	4.00c	0.00a	0.00b	66.00a	52.00a	6.00b	8.00b
	2014	82.00	82.00	40.00b	50.00	8.67c	12.00c	25.33a	28.00b	0.00a	8.00a	29.33b	40.00a	6.00b	16.00b
	LSD‡	17.993	14.253	21.918	24.151	24.247	29.284	14.952	14.374	0.000	5.900	20.683	27.631	18.28	17.862
	***P*-value**	0.1162	0.2236	0.0375	0.2595	<0.0001	0.0016	0.0096	<0.0001	.	0.0348	0.0003	0.0083	0.0007	0.0039
Richter 99 RY13C	**2009**	90.00	98.00	52.00b	65.00	54.00a	64.00a	14.00	13.00	2.00	4.50	10.00b	7.50b	34.00	31.50
	2012	94.00	89.78	68.00a	71.33	46.00a	53.33a	4.00	14.44	2.00	6.00	8.00b	2.22b	30.00	30.22
	2014	82.00	93.50	40.00b	56.00	8.00b	16.00b	12.00	8.00	4.00	0.00	40.00a	56.50a	30.00	36.00
	LSD‡	12.579	8.2103	14.67	20.803	14.453	17.621	14.453	13.182	8.7153	8.6697	8.7153	30.213	18.316	20.657
	***P*-value**	0.1491	0.1340	0.0046	0.3076	<0.0001	0.0002	0.3153	0.5519	0.8484	0.3262	<0.0001	0.0036	0.8615	0.8175
Ruggeri 140 RU354E	**2010**	98.00a	94.00	36.00	36.00b	80.00a	76.00a	14.00	24.00	0.00	4.00	26.00b	20.00b	8.00	14.00
	2014	76.00b	98.00	44.00	73.06a	16.00b	25.94b	0.00	12.22	2.00	0.00	48.00a	46.89a	4.00	16.44
	LSD‡	12.631	7.2922	18.157	22.712	18.734	21.598	15.64	14.553	4.612	5.6485	20.626	11.326	7.2922	19.714
	***P*-value**	0.0039	0.2415	0.3394	0.0055	<0.0001	0.0007	0.0729	0.0990	0.3466	0.1411	0.0393	0.0006	0.2415	0.7822

Table 2. Continued

Rootstock variety	Plant year	Total incidence* 2019	Total incidence* 2020	Botryosphaeriaceae 2019	Botryosphaeriaceae 2020	Celotheliaceae 2019	Celotheliaceae 2020	Diaporthaceae 2019	Diaporthaceae 2020	Diatrypaceae 2019	Diatrypaceae 2020	Hymenochaetaceae 2019	Hymenochaetaceae 2020	Togniniaceae 2019	Togniniaceae 2020
SO4 SO332A	**2011**	94.00	88.00b	26.00	32.00	74.00	66.00b	28.00	32.00	6.00	4.00	6.00	6.00	24.00a	22.00
	2012	94.00	98.00a	48.00	34.00	86.00	90.00a	22.00	36.00	0.00	0.00	8.00	6.00	0.00b	6.00
	LSD‡	13.045	9.7835	23.06	20.626	31.11	17.257	29.889	20.626	9.224	5.6485	14.584	13.045	15.64	17.862
	***P*-value**	1.0000	0.0462	0.0590	0.8287	0.3997	0.0125	0.6558	0.6666	0.1720	0.1411	0.7599	1.0000	0.0076	0.0727
US 8–7 UC274A	**2006**	88.00ab	100.00a	65.33ab	55.78	38.89b	88.00a	8.00b	12.22b	0.00b	4.22	53.78a	96.000a	10.22	55.78a
	2008	96.00a	89.56ab	84.00a	48.89	52.00ab	65.11b	10.00b	8.22b	0.00b	2.00	36.00a	40.67b	14.00	8.22b
	2012	86.00ab	92.00a	52.00bc	62.00	60.00a	64.00b	2.00b	8.00b	2.00b	0.00	6.00b	20.00c	12.00	16.00b
	2014	75.56b	76.00bc	55.11bc	56.00	6.00c	10.00c	28.44a	20.00ab	0.00b	8.00	0.00b	0.00d	6.22	10.00b
	2016	52.00c	72.00c	40.00c	52.00	4.00c	12.00c	12.00b	36.00a	8.00a	0.00	0.00b	4.00d	6.00	8.00b
	LSD‡	13.538	14.235	21.278	22.85	20.081	20.585	12.819	17.105	5.5972	7.7732	19.747	11.734	11.986	14.392
	***P*-value**	<0.0001	0.0029	0.0047	0.8036	<0.0001	<0.0001	0.0050	0.0139	0.0302	0.2049	<0.0001	<0.0001	0.5667	<0.0001

*Percentage incidence of fungal families in heads of rootstock mother vines following isolations from 50 vines per block.
† Values followed by the same letter do not differ significantly within a column of a cultivar.
‡ Least significant differences ($P = 0.05$) and *P*-values of each column per cultivar indicated in the two bottom rows.

year-to-year variation observed in several combinations (Supporting Information, Table S5, multivariate analyses).

3.7 Multivariate analysis

3.7.1 Principal component analysis

A significant positive correlation was observed between the percentage incidence of *D. seriata* in the heads of rootstock mother vines and the percentage incidence in one-year-old canes ($P < 0.0001$) as well as the DNA concentration in one-year-old canes originating from the same rootstock mother vines ($P = 0.010$) (Fig. 2, Supporting Information, Table S6). A significant positive correlation was also observed between the percentage incidence of *D. seriata* and *Pa. chlamydospora* in the heads of rootstock mother vines ($P = 0.045$). In contrast to these results, the percentage incidence of *Pa. chlamydospora* in one-year-old canes correlated negatively with its DNA concentration in the same one-year-old canes ($P = 0.0190$).

3.7.2 Multiple factor analysis

Results are presented in Fig. 3 and Supporting Information, Table S7. The percentage of vines containing black and brown wood streaking correlated positively with the percentage Celotheliaceae in the heads of rootstock mother vines ($P = 0.001$). The percentage of black or brown margins adjacent to necrotic wood correlated positively with the percentage Togniniaceae incidence in the heads of rootstock mother vines ($P = 0.033$). White rot correlated positively with the percentage Hymenochaetaceae and Togniniaceae incidences in the heads of rootstock mother vines ($P < 0.0001$ and $P = 0.007$), while Hymenochaetaceae and Togniniaceae incidences also correlated positively with one another ($P = 0.001$). White rot correlated negatively with the primary length of one-year-old canes (over all rootstocks) ($P = 0.015$). Black and brown necrosis or lines in or adjacent to white rot correlated positively with Botryosphaeriaceae incidence in the heads of mother vines ($P = 0.002$). The Celotheliaceae incidence correlated positively with wood streaking and the incidence of Togniniaceae in the heads of rootstock mother vines ($P = 0.001$, $P = 0.0002$). Black and brown necrosis or lines in or adjacent to white rot correlated positively with the incidence of Hymenochaetaceae and Togniniaceae in the heads of rootstock mother vines ($P < 0.0001$ and $P = 0.010$).

A significant positive correlation was observed between DNA concentration of *Pa. chlamydospora* and the minerals P, Mg, Fe, Cu, B and S, within one-year-old canes ($P = 0.020$, $P = 0.035$, $P = 0.002$, $P = 0.005$, $P = 0.021$ and $P = 0.008$, respectively), while a significant negative correlation was observed between *D. seriata* and K and Zn ($P = 0.042$ and $P = 0.038$, respectively). A significant negative correlation was observed between DNA concentration of *Pa. chlamydospora* and water content in one-year-old canes ($P = 0.002$).

The incidence of Botryosphaeriaceae in the heads of rootstock mother vines correlated negatively with mineral content including P, Ca, Mg, Na, Cu, S ($P = 0.00012$, $P = 0.012$, $P = 0.001$, $P = 0.001$, $P = 0.005$ and $P < 0.0001$) as well as with vine morphology including cane thickness, cane mass and primary and secondary cane lengths ($P = 0.007$, $P = 0.008$, $P = 0.006$ and $P = 0.039$). The Celotheliaceae incidence correlated negatively with starch content, N and K in one-year-old canes ($P = 0.009$, $P = 0.018$ and $P = 0.007$). The incidence of Diatrypaceae correlated negatively with cane morphology including thickness and mass ($P = 0.022$ and $P = 0.050$). The TPI correlated positively with the incidence of Botryosphaeriaceae and Hymenochaetaceae in

Table 3. The total number of isolations and percentage grapevine trunk disease pathogens isolated from five different symptom types

	Isolated pathogens							
Symptom	Botryosphaeriaceae	Celotheliaceae	Diaporthaceae	Diatrypaceae	Hymenochaetaceae	Togniniaceae	Total number of isolates	Total number of isolations
White rot, usually central	1.64*	1.67	0.22	0.02	30.78	1.99	1974	5436
Necrosis or lines in or adjacent to white rot	6.65	16.59	1.13	0.08	21.80	3.87	2538	5064
Black and brown wood streaking	3.87	17.14	1.21	0.21	1.28	1.80	7129	27 948
Sectorial or undefined necrosis	10.43	11.56	2.74	0.42	3.03	3.68	8209	25 776
Black or brown margins adjacent to necrotic wood	12.47	9.91	2.76	0.46	4.20	4.67	1263	3664
Unknown	7.73	11.55	1.91	0.38	3.15	3.15	292	1048
Total number of isolates	4734	9184	1233	193	4101	1960	21 405	68 936

*The numbers represent the percentage of isolates of a specific fungal family from the total number of isolations that were made from a specific symptom type.

the heads ($P = 0.001$ and $P = 0.004$). The Diaporthaceae incidence correlated negatively with starch content in the one-year-old canes ($P = 0.027$).

Noteworthy observations between minerals and cane morphology included a significant negative correlation between K and cane thickness, −mass, −primary, and secondary cane length ($P = 0.026$, $P = 0.002$, $P = 0.049$ and $P = 0.0001$), as well as the significant positive correlation between both cane mass and primary cane length with Cu ($P < 0.0001$ and $P = 0.0001$).

4 DISCUSSION

This study investigated the effect of rootstock mother block age on fungal infection status, vine physiology and cane morphology, as well as the interactions among these variables. Multivariate analysis revealed planting year and season to have inconsistent effects on the data, suggesting that vine physiology and cane morphology are not directly influenced by the age of the rootstock mother block. However, upon further investigation, certain correlations frequently occurred between morphological, physiological and infection variables. High incidences of GTD pathogens were recorded in the heads of all rootstock mother vines, while much lower incidences were recorded in one-year-old rootstock canes. The qPCR analysis revealed incrementally higher positive samples in one-year-old canes than recorded with traditional isolation methods.

Pathogens of all major GTDs were isolated from the heads of mother vines in certified rootstock mother blocks. Incidences of up to 90.00% of Celotheliaceae, of which *Pa. chlamydospora* was the only identified species, were reported in the heads of an eight-year-old mother block and at 30.00% in a block as young as 4-years-old. The detection of such high incidences of GTDs in relatively young blocks is concerning, as it suggests early pathogen establishment within rootstock mother blocks. Celotheliaceae was the main isolated fungal family from both black and brown wood streaking as well as sectorial or undefined necrosis and isolated 17.14% and 11.56%, respectively, which is consistent with previous studies.[87–93] The positive correlation observed between the Celotheliaceae and wood streaking shows that *Pa. chlamydospora* is the primary cause of this symptom type.[88,94–97]

The negative correlation observed between the DNA concentration of *Pa. chlamydospora* and water content indicates that *Pa. chlamydospora* colonization is higher in water-stressed cane material, or that the stress has been caused by the infection, as shown in previous studies.[98,99] The negative correlation of *Pa. chlamydospora* with starch content in the one-year-old canes can be ascribed to this pathogen utilizing the starches within the vines.[49,81,100] 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,[101] which is a cost-intensive mechanism.[102] The result of these disruptions is a reduced reservoir of carbon reserves, which could potentially lead to a decline in plant growth and vitality of the vine in the following year.[49]

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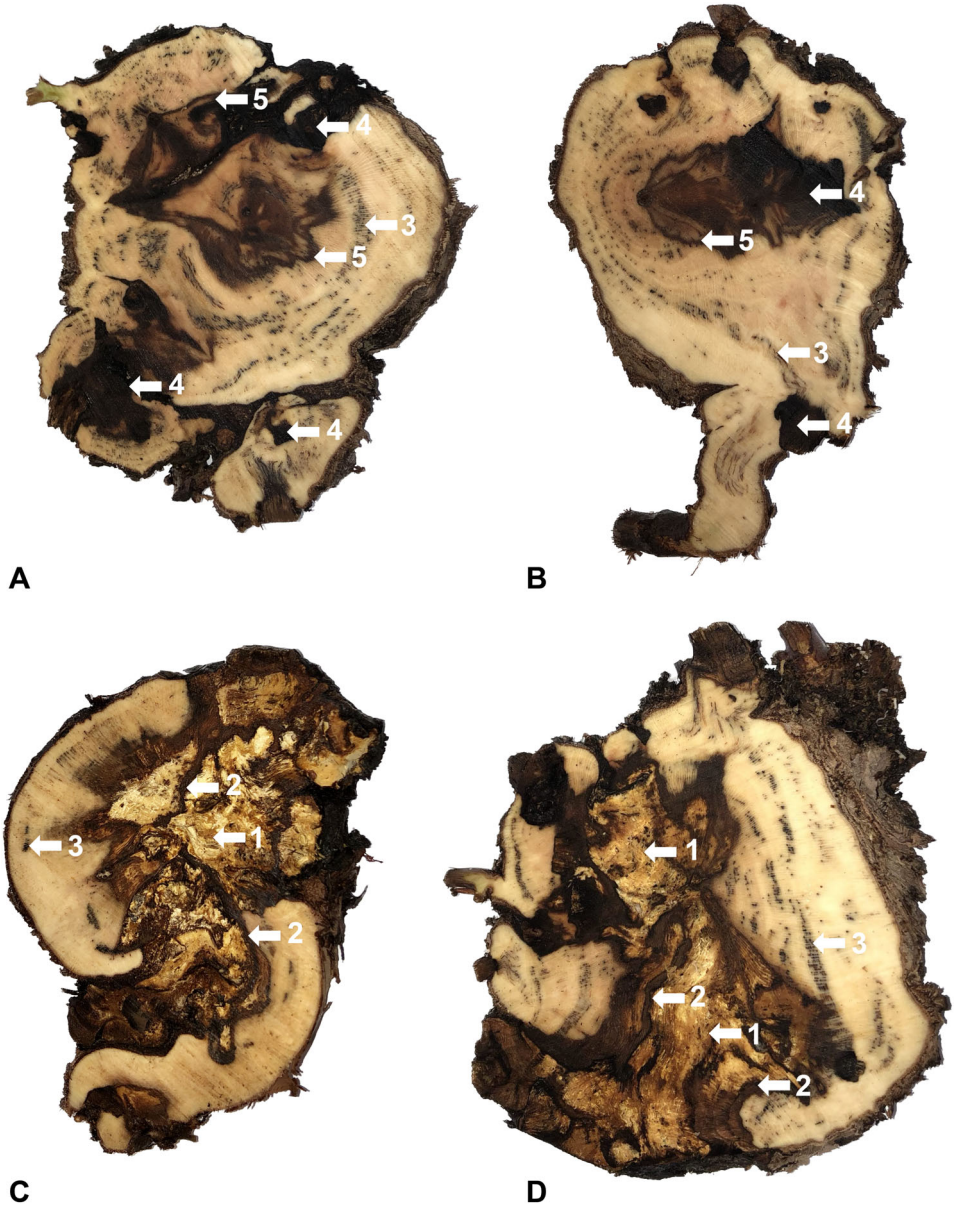

Figure 1. (A–D) Symptoms observed in the heads of certified rootstock mother vines in the Wellington region of South Africa. 1) White rot, usually central, 2) black and brown necrosis or lines in or adjacent to white rot, 3) black and brown wood streaking, 4) sectorial or undefined necrosis and 5) black or brown margins adjacent to necrotic wood.

Phaeomoniella chlamydospora is also associated with the chronic form of esca and is considered by some to be the pioneering pathogen of the disease,[5,87,103,104] predisposing the vines to infection by the wood rotting fungi in the Hymenochaetaceae.[3,87,88,105,106] The dominant Hymenochaetaceae species were *F. capensis* and *Fomitiporella* sp. (taxon 1). Generally, Hymenochaetaceae was more consistently isolated from older rootstock mother blocks, which is in accordance with White *et al.*[86] However, Hymenochaetaceae species were isolated from rootstock mother blocks as young as 3-years-old (Paulsen 1103 PS28I with 8.00% incidence in the 2019 season). In this study, 30.78% of isolations made from white rot yielded Hymenochaetaceae species, while 21.80% of isolations made from necrosis or lines in or adjacent to white rot yielded Hymenochaetaceae species. The Hymenochaetaceae was also isolated from sectorial or undefined necrosis, black and brown wood streaking and black or brown margins adjacent to necrotic wood, which is consistent with Sparapano *et al.*[107] who showed Hymenochaetaceae to induce brown discoloration in grapevine wood during the initial stages of colonization.

The occurrence of Esca-associated pathogens in young mother vines is of critical importance as these pathogens can significantly reduce their lifespan. Liminana *et al.*[39] investigated Esca in rootstock mother vines and showed that the necrotic area percentage correlates significantly with vine mortality and white rot incidence. Moreover, the negative correlation between white rot in the heads of mother vines and the primary length of one-year-old canes indicated that white rot leads to shorter cane production, thereby significantly reducing rootstock mother block yields and affecting income.

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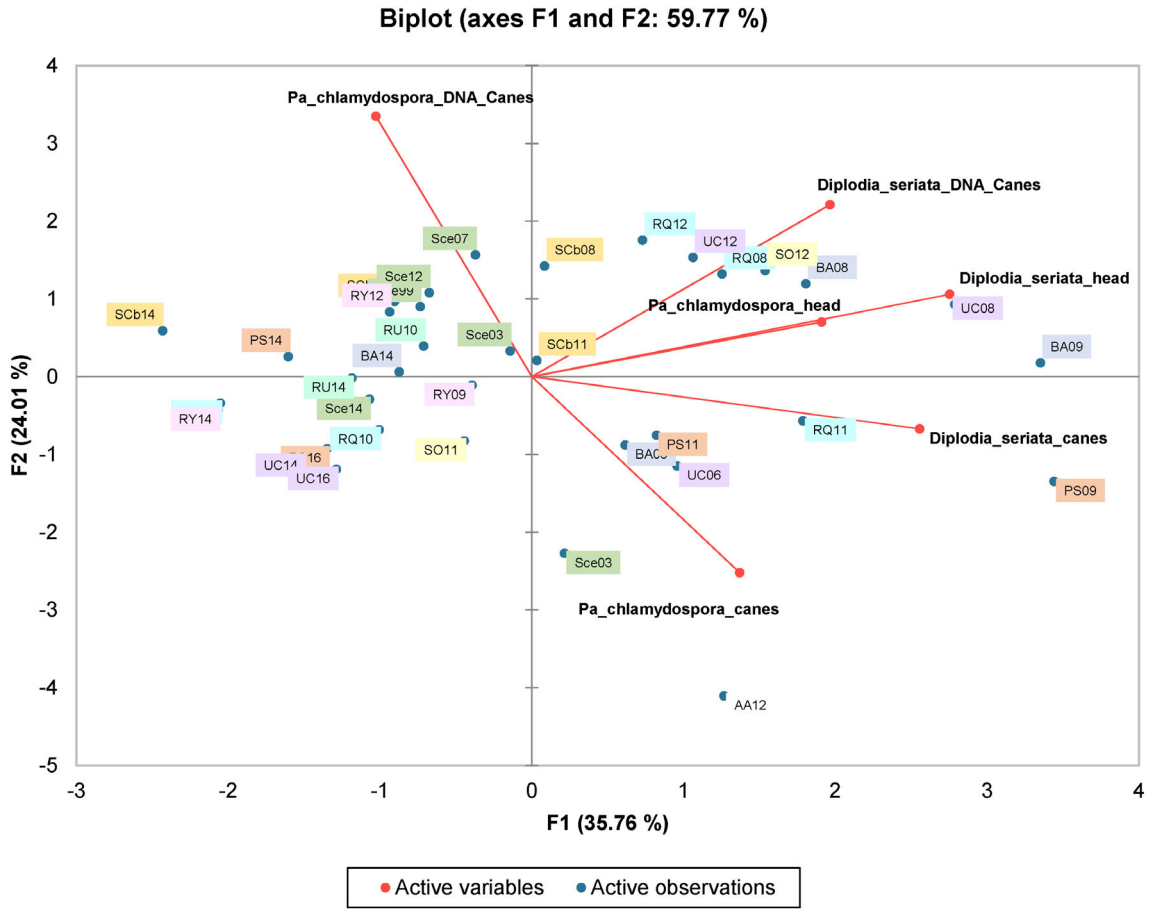

Figure 2. Principle component analysis (PCA) of all variables included in the 2019 season. The active variables include the incidence and DNA concentrations of *Phaeomoniella chlamydospora* and *Diplodia seriata* in one-year-old canes, as well as the incidence of *Pa. chlamydospora* and *D. seriata* in the heads, while the active observations include the rootstock variety and planting year (BA – Millardet et Grasset 143 B BA31C; PS – Paulsen 1103 PS28I; SCb – Ramsey SC18AB; Sce – Ramsey SC18AE; RQ – Richter 110 RQ28C; RY – Richter 99 RY13C; RU – Ruggeri 140 RU354E; SO – Selektion Oppenheim 4 SO332A; UC – US VIT 8–7 UC274A).

The causal agents of Botryosphaeria dieback were the second most isolated pathogens from heads of rootstock mother vines. The predominant identified species were *D. seriata*, while *N. parvum*, *Diplodia mutila*, *Lasiodiplodia plurivora*, *Neofusicoccum australe*, *Diplodia pterocarpi*, *Lasiodiplodia mahajangana* and *Neofusicoccum 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.[108,109] Symptoms are usually observed in vineyards of 8 years and older,[110] although cankers, dieback, and plant death have been recorded in 3- to 5-year-old table grape vines.[111] Whitelaw-Weckert *et al.*[17] 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 the heads of a 3-year-old rootstock mother block, while incidences as high as 84.00% have been recorded in an 11-year-old block. A recent study by Larach *et al.*[112] 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 spores were trapped from late autumn to early spring in South Africa,[113] which coincides with the harvesting period of canes (autumn) from rootstock mother vines, explaining the high incidences. Additionally, research has demonstrated that these fungi have the capability to infect healthy plants directly through lenticels, stomata, or other openings,[114,115] facilitated by the presence of inoculum on the bark of rootstock cuttings obtained from the mother vine.[48] The negative correlation observed between incidence of Botryosphaeriaceae and cane morphology shows that it directly impacts the development of one-year-old canes, likely due to fungal metabolites being produced by these fungi that result in structural modifications of the canes, specifically that of suberin deposition[116] 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 suggest that Botryosphaeriaceae species likely co-occur in Esca-associated symptoms. Botryosphaeriaceae species are known to be associated with Esca-diseased vines,[88] although their specific role remains uncertain.[91] In the past, these fungi have been isolated from various sources with Esca-related symptoms, with a higher frequency in cases of sectorial necrosis.[37,87,88] Multiple trunk pathogens and other wood-colonizing fungi may interact in the process of wood decomposition.[117] This can result in facilitative interactions among them,[118] which is more likely in communities harboring more species. Furthermore, in the

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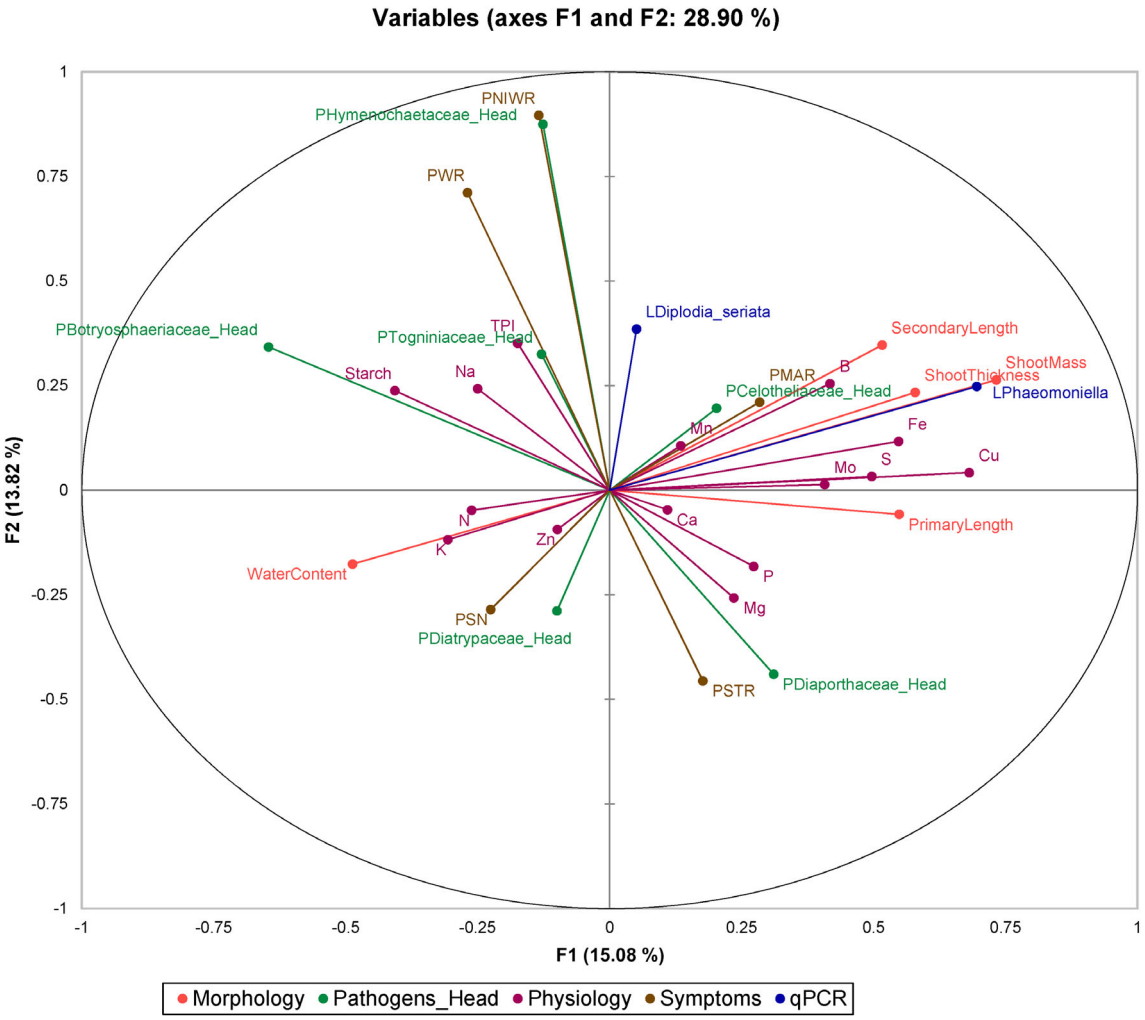

Figure 3. Variable loading plot for multiple factor analysis of all variables included in the 2019 and 2020 seasons. The morphology data are presented in red, the physiology data in purple, the grapevine trunk disease incidence in the heads of the mother vines in green, the symptom types in the heads of the mother vines in brown (PWR – percentage white rot, usually central; PNIWR – percentage necrosis or lines in or adjacent to white rot; PSTR – percentage black and brown wood streaking; PSN – percentage sectorial or undefined necrosis; PMAR – percentage black or brown margins adjacent to necrotic wood), and DNA concentrations of *Phaeomoniella chlamydospora* and *Diplodia seriata* in one-year-old canes in blue.

presence of multiple GTD fungi within a grapevine, the threshold for resource utilization could be reached more quickly.[119]

The positive correlation between the incidences of *Pa. chlamydospora* and *D. seriata* in the heads of rootstock mother vines is consistent with results obtained in other studies. Spagnolo *et al.*[120] and Elana *et al.*[121] reported infections by Botryosphaeriaceae species and *Pa. chlamydospora* to frequently co-occur in propagation material, albeit *Pa. chlamydospora* showed a slower colonization rate than *D. seriata*.[121] Both Botryosphaeriaceae species and *Pa. chlamydospora* have been shown to produce the phytotoxic metabolites iso-sclerone and exopolysaccharides[122–124] and could result in a synergistic action.[120]

The positive correlation between TPI in one-year-old canes and the incidence of Botryosphaeriaceae and Hymenochaetaceae in the heads of rootstock mother vines suggests that infection by these pathogens results in more severe reactions, likely due to a locally induced response. Wallis *et al.*[125] associated *D. seriata* with significantly higher concentrations of phenolic compounds, while this species was shown to display high sensitivity to piceatannol, pterostilbene, and ε-viniferin.[126] However, it is important to note that even though overall TPI content yielded these results, measurements may obscure the ecologically significant individual compounds.[55,127,128]

In this study, only five Diaporthaceae species were identified from grapevine rootstock mother blocks, predominantly *D. foeniculina* and *D. ampelina*. In contrast, 19 Diaporthaceae species have been reported from commercial vineyards in South Africa.[67] Diaporthaceae species occurred mainly in sectorial or undefined necrosis and black and brown wood streaking areas, consistent with previous reports that associated it with perennial trunk cankers.[129–131]

Much lower incidences of GTDs were recorded following fungal isolations from one-year-old rootstock canes, which correlated poorly with rootstock mother block age. 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. Detectable concentrations of *D. seriata* DNA were recorded in 46.15%, 38.00% and 77.37% of all surveyed zones in the respective seasons. In 2015, Billones-Baaijens *et al.*[48] suggested dormant cuttings' bark as a potential infection source for Botryosphaeriaceae species. In 2018, Waite *et al.*[19] confirmed epiphytic inoculum's ability to penetrate cane

bark and cause infections. The positive correlation observed between *D. seriata*'s incidences and DNA concentration in one-year-old canes and incidences in the heads of rootstock mother vines indicates that higher levels of infection in the heads result in more contaminated one-year-old canes, likely due to active mycelial growth from the heads of infected mother vines as described by Fourie and Halleen.[33] Amponsah *et al.*[132] reported that hyphae in xylem of grapevine stems inoculated with species of Botryosphaeriaceae moved faster acropetally than basipetally. The authors reisolated *Neofusicoccum luteum* 60–70 cm acropetally from the inoculation site after 4 months, by which time the pathogen had progressed into lateral shoots. In a study by Billones-Baaijens *et al.*[43] 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.*[17] isolated *D. seriata* from 25% of canes sampled from rootstock mother blocks.

Despite *Pa. chlamydospora* occurring at low incidences in rootstock canes when recorded using isolation techniques, 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 vines. Most canes were considered incidence-free when determining infection levels of *Pa. chlamydospora* using isolation methods, similar to Zanzotto *et al.*[27] who were unable to isolate *Pa. chlamydospora* from suspected infected cutting material. However, Fourie and Halleen[29] could isolate this pathogen from South African grapevine material, albeit in very limited quantities. In contrast, detectable levels of *Pa. chlamydospora* DNA were recorded in 98.97%, 99.50% and 71.05% of all surveyed zones in the respective seasons, with the highest *Pa. chlamydospora* DNA concentration in a relatively young rootstock mother block, at 1055.25 ng μL^{-1}. Bruez *et al.*[133] reported *Pa. chlamydospora* to be the most abundant species in non-necrotic tissues from healthy plants, suggesting a possible non-pathogenic endophytic behavior. The negative correlations observed between the incidence of *Pa. chlamydospora* and DNA concentrations thereof in one-year-old canes can likely be ascribed to inoculum nestled beneath the bark of the canes, which is killed during the process of surface sterilization 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.[134] This external inoculum may be introduced to sprawled canes directly from infested soils[36,38,135] as reported for bark at soil level.[136] The negative correlations may also show the difficulty of isolating this species from wood, even though DNA in the form of mycelia or spores is present within the canes. Retief *et al.*[34] found up to four times more positive detections of *Pa. chlamydospora* with qPCR when compared to traditional isolation techniques. These results indicate that one-year-old canes from rootstock mother blocks can harbor GTD inoculum, either systemically or as inoculum underneath the bark, which serves as source of infection in both the grapevine nurseries as well as in newly established vineyards.

Correlations were also observed between mineral content and cane morphology, notably a significant positive relationship between Cu and both cane mass and primary cane length. This may reflect the indirect effects of Cu-containing sprays, which promote overall plant health and growth. Similarly, N, K, and P are key fertilizer components that support cane development by replenishing minerals lost during harvest.

5 CONCLUSION

This study demonstrated the detrimental impact of GTD infections on cane production in rootstock mother blocks. One-year-old canes were identified as a primary inoculum source for *Pa. chlamydospora* and *D. seriata*. Nevertheless, when industry-standard canes are selected for propagation, vine physiology and morphology appear largely unaffected by mother block age. Instead, these parameters are more strongly influenced by rootstock variety and seasonal effects. With appropriate management of aging rootstock mother blocks, as emphasized by Waite *et al.*[19] and Smart,[137] cane physiology and morphology are unlikely to compromise grafting success, provided that canes are free of latent GTD infections.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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