Eutypa, *Eutypella*, and *Cryptovalsa* Species (Diatrypaceae) Associated with *Prunus* Species in South Africa

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Abstract

Stone fruit trees (*Prunus* spp.) are economically important fruit trees cultivated in South Africa. These trees are often grown in close proximity to vineyards and are to a large extent affected by the same trunk disease pathogens as grapevines. The aim of the present study was to determine whether stone fruit trees are inhabited by Diatrypaceae species known from grapevines and whether these trees could act as alternative hosts for these fungal species. Isolations were carried out from symptomatic wood of *Prunus* species (almond, apricot, cherry, nectarine, peach, and plum) in stone fruit growing areas in South Africa. Identification of isolates was based on phylogenetic analyses of the internal transcribed spacer region and β -tubulin gene. Forty-six Diatrypaceae isolates were

obtained from a total of 380 wood samples, from which five species were identified. All five species have also been associated with dieback of grapevine. The highest number of isolates was found on apricot followed by plum. No Diatrypaceae species were isolated from peach and nectarine. *Eutypa lata* was the dominant species isolated (26 isolates), followed by *Cryptovalsa ampelina* (7), *Eutypa cremea* (5), *Eutypella citricola* (5), and *Eutypella microtheca* (3). First reports from *Prunus* spp. are *E. cremea*, *E. citricola*, and *E. microtheca*. Pathogenicity tests conducted on apricot and plum revealed that all these species are pathogenic to these hosts, causing red-brown necrotic lesions like those typical of *Eutypa* dieback on apricot.

Prunus species are widely cultivated in different parts of the world. Members of the genus *Prunus* are commonly referred to as stone fruits and include *Prunus armeniaca* L. (apricot), *P. avium* L. (sweet cherry), *P. domestica* L. (European plum), *P. dulcis* (Mill.) D.A. Webb (almond), *P. persica* (L.) Batsch (peach), *P. persica* var. *nucipersica* (Suckow) C.K. Schneid. (nectarine), and *P. salicina* Lindl. (Japanese plum). In South Africa, stone fruits are cultivated on 22% of the total area of land planted with fruit trees (18,025 ha) and the Western Cape Province is the most significant producer of these deciduous fruits, with over 90% (16,447 ha) of the country's stone fruit production area (Hortgro 2015). In the Western Cape Province, stone fruit production predominantly occurs in districts such as Klein Karoo, Ceres, Paarl, Piketberg, and Stellenbosch. Provinces in South Africa with minor stone fruit production include Limpopo (529 ha), Eastern Cape (596 ha), Mpumalanga (160 ha), Free State (119 ha), and Gauteng (68 ha) (Hortgro 2015).

Japanese plums dominate the stone fruit industry of South Africa in terms of production and exports (Potelwa et al. 2014). Over 81,000 tonnes of plums were produced in the 2014–15 season from 5,205 ha land, 7% more than in the 2013–14 season. Seventy-four percent (over 60,000 t) of all the plums produced in South Africa are exported (Hortgro 2015). South Africa is the leading producer of apricots in the southern hemisphere but apricots are ranked second in terms of production after plums (Potelwa et al. 2014). In 2014–15, 57,982 t of apricot were produced from 2,888 ha land, 19% more than in the 2013–14 season (Hortgro 2015). Peaches (including dessert and cling) and nectarines are also grown in large areas (7,434 and 2,229 ha, respectively), but only 5% of these fruits are exported (Hortgro 2015; Potelwa et al. 2014). Other stone fruits cultivated on a smaller scale in South Africa include cherries and prunes whose

production in the 2014–15 season was 282 and 2,219 t, respectively (DAFF 2016). Almonds are also cultivated on a very small scale but no formal statistics about the South African almond industry is available. The bulk of stone fruit is absorbed by the European and Russian markets followed by the Middle East (Potelwa et al. 2014). The stone fruit industry has a turnover of approximately US \$178.4 million and generates 22% of the fruit industry income (Hortgro 2015) and thus, it is a significant contributor to the economy of South Africa.

Dieback and canker diseases are generally a threat to productivity because they reduce the lifespan and yields of fruit trees. Dieback of stone fruit trees is a common phenomenon in South Africa (Heyns 1960; Roos and Hattingh 1983). Dieback symptoms are usually observed in spring and they include the failure of buds to sprout, chlorotic and stunted foliage, and cankers as well as gums exuding from the observed cankers. Root systems of dying trees are, however, not affected (Heyns 1960). These aforementioned symptoms have been ascribed to a number of factors including the bacterial pathogen *Pseudomonas syringae* van Hall (Heyns 1960; Roos and Hattingh 1983) and climatic conditions (Stassen and van Zyl 2014). However, these symptoms are also typical of infection by fungi like *Eutypa lata* (Pers.) Tul. & C. Tul. *E. lata* (Ascomycota, Diatrypaceae) was first described as the causal agent of gummosis or dieback disease of apricot in Australia (Carter 1957) and has since been known to be responsible for dieback of several stone fruit tree species (Carter 1982, 1995; Matthee et al. 1974; Munkvold and Marois 1991, 1994; Rumbos 1997), grapevine (Ferreira 1987; Moller and Kasimatis 1978; Sosnowski et al. 2007), and other woody hosts worldwide (Rumbos 1986; Tosi and Natalini 2009; Wenneker et al. 2011).

The Diatrypaceae consists of many species and genera that colonize many woody hosts as saprophytes and pathogens. Recently, this family has received considerable attention because of the discovery of several of its species on grapevines that were affected by dieback and cankers in different grape growing regions of the world (Díaz et al. 2011; Luque et al. 2012; Paolinelli-Alfonso et al. 2015; Pitt et al. 2010; Rolshausen et al. 2014; Trouillas and Gubler 2004; Trouillas et al. 2010, 2011). In South Africa, investigations of the diversity of Diatrypaceae species associated with dieback and canker symptoms on grapevine, resulted in the identification of seven species (Moyo et al. 2018). In South Africa, vineyards are usually established in close proximity to stone fruit orchards. Other known grapevine trunk disease pathogens, for example several Botryosphaeriaceae and *Phaeoacremonium* spp.,

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have previously been detected in stone fruit orchards adjacent to vineyards in South Africa (Damm et al. 2007, 2008), apparently disseminated by an inoculum exchange between both hosts. It is therefore possible that spores of Diatrypaceae species are dispersed from grapevine to stone fruit trees or vice versa.

The only Diatrypaceae species reported to occur on stone fruits in South Africa are *E. lata* and *Cryptovalsa ampelina* (Nitschke) Fuckel (Damm et al. 2009; Matthee et al. 1974). In light of the new Diatrypaceae species discovered on dieback-affected grapevines in South Africa (Moyo et al. 2018), it is a possibility that stone fruit trees serve as additional hosts for the survival of these pathogens in the absence of vineyards and could also be sources of inoculum to adjacent vineyards. The aim of this study was, therefore, to determine whether

stone fruit trees harbor Diatrypaceae species known to occur on grapevine as well as to investigate the importance and contribution of Diatrypaceae species in dieback and cankers of stone fruits. The objectives of this study were to (1) determine the diversity of Diatrypaceae species occurring on stone fruit trees in South Africa, and (2) conduct pathogenicity tests of Diatrypaceae species on plum and apricot, which are the first and second most produced stone fruits in South Africa, respectively.

Materials and Methods

Sampling. Wood of different *Prunus* spp. (plum, peach, nectarine, apricot, sweet cherry, and almond) was sampled in stone fruit orchards in climatically different table and wine grape producing areas in the

Table 1. List of isolates (representative) collected in this study and reference strains used for phylogenetic analysis

Species	Host	Origin	Strain number	GenBank number	
				ITS	β-tubulin
<i>Cryptosphaeria ligniotea</i> ^z	<i>Populus tremula</i>	Switzerland	CBS 273.87	KT425233	KT425168
<i>Cryptosphaeria multicontinentalis</i>	<i>Populus balsamifera</i>	Australia	NSW03PO	KT425237	KT425172
<i>Cryptosphaeria pullmanensis</i> ^z	<i>Populus trichocarpa</i>	U.S.A.	ATCC 52655	KT425235	KT425170
<i>Cryptovalsa ampelina</i>	<i>Fraxinus latifolia</i>	U.S.A.	DMO 100	GQ293908	GQ293978
<i>Cryptovalsa ampelina</i>	<i>Vitis vinifera</i>	Australia	HVVIT04	HQ692558	HQ692459
<i>Cryptovalsa ampelina</i>	<i>Prunus salicina</i>	South Africa	STEU 5947, CBS 121429	KY752771	KY752799
<i>Cryptovalsa ampelina</i>	<i>Prunus armeniaca</i>	South Africa	STEU 6087, CBS 120836	KY752772	KY752800
<i>Cryptovalsa ampelina</i>	<i>Prunus armeniaca</i>	South Africa	STEU 7942	KY752773	KY752798
<i>Cryptovalsa rabenhorstii</i>	<i>Vitis vinifera</i>	Australia	WA07CO	HQ692620	HQ692522
<i>Eutypa consobrina</i>	<i>Arundo donax</i>	Spain	F-091.961	AJ302447	...
<i>Eutypa cremea</i>	<i>Arundo donax</i>	France	CBS 286.87	AY684225	AY684203
<i>Eutypa cremea</i>	<i>Prunus salicina</i>	South Africa	STEU 5832, CBS 120837	KY752762	KY752791
<i>Eutypa cremea</i>	<i>Prunus armeniaca</i>	South Africa	STEU 6085, CBS 121432	KY752763	KY752792
<i>Eutypa cremea</i>	<i>Prunus armeniaca</i>	South Africa	STEU 7945	KY752761	KY752801
<i>Eutypa cremea</i>	<i>Prunus armeniaca</i>	South Africa	STEU 8139	KY752764	KY752790
<i>Eutypa cremea</i>	<i>Prunus armeniaca</i>	South Africa	STEU 8410	KY752765	KY752789
<i>Eutypa cremea</i> ^z	<i>Vitis vinifera</i>	South Africa	STEU 8082	KY111656	KY111598
<i>Eutypa crustata</i>	<i>Ulmus</i> sp.	France	CBS 210.87	AJ302448	DQ006968
<i>Eutypa laevata</i>	<i>Salix</i> sp.	Switzerland	CBS 291.87	HM164737	HM164771
<i>Eutypa lata</i>	<i>Prunus armeniaca</i>	South Africa	STEU 6081	KY752768	KY752793
<i>Eutypa lata</i>	<i>Prunus armeniaca</i>	South Africa	STEU 6082, CBS 121430	KY752766	KY752794
<i>Eutypa lata</i>	<i>Prunus armeniaca</i>	South Africa	STEU 6083	KY752767	KY752795
<i>Eutypa lata</i>	<i>Prunus salicina</i>	South Africa	STEU 6084, CBS 121431	KY752769	KY752796
<i>Eutypa lata</i>	<i>Vitis vinifera</i>	U.S.A.	EAMS100	HM164730	HM164764
<i>Eutypa lata</i>	<i>Populus nigra</i> ‘italica’	Australia	SAPN01	HQ692616	HQ692500
<i>Eutypa lata</i>	<i>Prunus armeniaca</i>	South Africa	STEU 8411	KY752770	KY752797
<i>Eutypa lata</i> var. <i>aceri</i>	<i>Acer campestre</i>	France	CBS 217.87	HM164734	HM164768
<i>Eutypa lejoplaca</i>	<i>Acer pseudoplatanus</i>	Switzerland	020202-5	AY684221	AY684196
<i>Eutypa leptoplaca</i>	<i>Frangula alnus</i>	Switzerland	CBS 287.87	AY684226	AY684204
<i>Eutypa leptoplaca</i>	<i>Cissus hypoglauca</i>	Australia	CBS 288.87	AY684227	AY684205
<i>Eutypa petrakii</i> var. <i>petrakii</i>	<i>Prunus spinosa</i>	Switzerland	CBS 244.87	HM164735	HM164769
<i>Eutypa sparsa</i>	<i>Populus</i> sp.	Switzerland	3802-3a	AY684200	AY684219
<i>Eutypa tetragona</i>	<i>Laburnum alpinum</i>	Switzerland	190802-3	AJ684223	AY684202
<i>Eutypa tetragona</i>	<i>Sarothamnus scoparius</i>	France	CBS 284.87	DQ006923	DQ006960
<i>Eutypella australiensis</i> ^z	<i>Acacia longifolia</i> subsp. <i>sophorae</i>	Australia	CNP03	HM581945	HQ692479
<i>Eutypella citricola</i>	<i>Citrus paradisi</i>	Australia	HVGRF01	HQ692589	HQ692521
<i>Eutypella citricola</i>	<i>Citrus sinensis</i>	Australia	HVOT01	HQ692581	HQ692509
<i>Eutypella citricola</i>	<i>Vitis vinifera</i>	Australia	HVVIT07	HQ692579	HQ692512
<i>Eutypella citricola</i>	<i>Prunus armeniaca</i>	South Africa	STEU 5948, CBS 120838	KY752778	KY752785
<i>Eutypella citricola</i>	<i>Prunus armeniaca</i>	South Africa	STEU 7943	KY752779	KY752782
<i>Eutypella citricola</i>	<i>Prunus salicina</i>	South Africa	STEU 7944	KY752777	KY752784
<i>Eutypella citricola</i>	<i>Prunus salicina</i>	South Africa	STEU 8249	KY752781	KY752802
<i>Eutypella citricola</i>	<i>Prunus armeniaca</i>	South Africa	STEU 8412	KY752780	KY752783
<i>Eutypella microtheca</i>	<i>Ulmus procera</i>	Australia	ADEL300	HQ692560	HQ692528
<i>Eutypella microtheca</i> ^z	<i>Citrus paradisi</i>	Australia	HVGRF02	HQ692569	HQ692533
<i>Eutypella microtheca</i>	<i>Vitis vinifera</i>	Australia	T2R2S7	HQ692566	HQ692532
<i>Eutypella microtheca</i>	<i>Prunus salicina</i>	South Africa	STEU 5949, CBS 120839	KY752776	KY752787
<i>Eutypella microtheca</i>	<i>Prunus armeniaca</i>	South Africa	STEU 6086, CBS 121433	KY752774	KY752788
<i>Eutypella microtheca</i>	<i>Prunus armeniaca</i>	South Africa	STEU 7941	KY752775	KY752786
<i>Eutypella vitis</i>	<i>Vitis labrusca</i>	U.S.A.	ATCC 64171	AJ302466	...
<i>Eutypella vitis</i>	<i>Vitis vinifera</i>	U.S.A.	MSUELM13	DQ006943	DQ006999

^z Type strains. Sequences generated in this study are emphasized in bold face.

Western Cape (Ceres, Stellenbosch, Paarl, Franschhoek, Robertson, Bonnievale, Calitzdorp, Montagu) and Limpopo Province (Mookgophong and Modimolle) in South Africa during 2004, 2005, 2013, 2014, and 2015. Additional wood samples of *Prunus* spp. were also collected from small orchards or gardens located in grapevine producing areas of the Western Cape of South Africa. Wood samples were obtained mainly from living branches with symptoms such as dieback, cankers, necrosis, and gummosis.

Fungal isolation. Collected wood samples were taken to the laboratory where they were cut into smaller pieces to reveal internal symptoms. These pieces were then surface sterilized by immersing in 70% ethanol for 30 s followed by 2 min in 3.5% sodium hypochlorite and 30 s in 70% ethanol. During isolation, 12 small pieces (~1 × 1 mm) were cut from the margin between necrotic and apparently healthy tissue and transferred to potato dextrose agar (PDA, Biolab, South Africa) amended with chloramphenicol (250 mg/liter) (PDA-C). The plates with wood pieces were incubated at 24°C for 4 weeks with approximately 12 h of daylight and 12 h of darkness. The plates were frequently checked and isolates resembling *Diatrypaceae* species (based on their white to white-cream, cottony, slow-growing mycelium on PDA) were transferred onto new PDA-C plates for further identification. Collected isolates are maintained in the culture collections of the Department of Plant Pathology, University of Stellenbosch, South Africa (STEU) and Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands (CBS).

In order to obtain fungal isolates from fruiting bodies, dead wood of stone fruit trees with black, charcoal-like stroma with fruiting

bodies resembling those of *E. lata* was also collected from the floor of orchards. Depending on availability of such wood, one to three samples per orchard were randomly collected from the different sampling areas. Isolations and fungal identification from collected samples was achieved as described by Moyo et al. (2018).

DNA sequence analysis. DNA extraction from fungal mycelium was conducted as outlined by Moyo et al. (2016). Two gene regions were amplified, sequenced, and used for phylogenetic analyses to determine the identity of fungal isolates obtained from wood necroses. The 5.8S ribosomal gene with the two flanking internal transcribed spacers, ITS1 and ITS2 (ITS), and partial β -tubulin gene (TUB2) were amplified for all isolates of interest using primer pairs ITS1/ITS4 (White et al. 1990) and Bt2a/Bt2b (Glass and Donaldson 1995), respectively. The conditions, procedures for PCR, and sequencing were the same as those described by Moyo et al. (2018). Sequences of representative isolates were used in the phylogenetic analysis and deposited in GenBank (Table 1).

The phylogenetic analysis of the individual ITS and TUB2 sequences, as well as of the combined datasets (ITS and TUB2) were performed using maximum likelihood (ML). The ITS and TUB2 sequences, respectively, of isolates obtained in this study were aligned with sequences of *Diatrypaceae* retrieved from GenBank as well as the outgroup *Hypoxylon fragiforme* (Pers.) J. Kickx f. Sequences were aligned with MAFFT v7.017 (Katoh et al. 2002) in Geneious v8.1 (<https://www.geneious.com>, Kearse et al. 2012), using the default parameters. Maximum likelihood analyses were performed using PhyML (Guindon and Gascuel 2003)

Fig. 1. Dieback symptoms observed on stone fruit trees in South Africa. **A**, Dead plum tree between living trees in an orchard. **B**, Canker on the trunk of a plum tree in an orchard. **C**, Black/brown discoloration extending from the pruning wound of a plum shoot. **D**, Canker and gummosis on an apricot shoot. **E**, Wedge shaped canker on an apricot branch. **F**, Brown discoloration associated with a canker on apricot wood. **G**, Brown/black spots and necrosis extending from a wound (arrow) on apricot wood. **H**, Branch dieback on a cherry plant in an orchard. **I**, Gummosis on a cherry branch. **J**, Cankers associated with a pruning wound (arrow) on a cherry tree. **K**, Discoloration extending from pruning wound (arrow) of a cherry shoot. **L**, Canker associated discoloration of almond wood. **M**, Brown/black discoloration of almond wood.

in Geneious v8.1 under the general time reversible (GTR) model. Both the proportion of invariable sites and gamma distribution parameter were estimated and bootstrap support values were calculated from 1,000 replicates.

Pathogenicity on detached shoots. Preliminary pathogenicity tests were conducted with eight Diatrypaceae isolates on detached apricot (cv. Bulida) and plum (cv. Southern Bell) shoots. One or two isolates of each of the five species of Diatrypaceae, depending on the availability of isolates at the time of this experiment, were used and treated as subsamples in statistical analysis. Vegetative shoots were collected shortly after harvest, cut into 12 cm pieces (5 to 8 mm diam.), and surface sterilized by immersing in 0.1% solution of a patented didecyldimethylammonium chloride formulation (Sporekill, ICA International Chemicals Pty. Ltd., Stellenbosch, South Africa) for 40 s. Wounds were then made with a 4-mm cork borer and shoots were inoculated with agar plugs colonized by 2-week-old fungal mycelium. Uncolonized PDA plugs were used as controls. Each treatment combination consisted of one shoot per host, which was replicated four times in each of three blocks (= moist chambers). The layout of the pathogenicity trial was a randomized block design. All inoculated wounds were covered with Parafilm and shoots were incubated at 25°C in moist chambers (>93% RH) for 2 weeks. Thereafter, the shoots were split longitudinally through the inoculation point and the internal discoloration (referred here as lesions) measured from both directions of the inoculation point. The data on lesion length was subjected to one-way analysis of variance (ANOVA) using General Linear Model in SAS version 9.2 (SAS Institute, Cary, NC). Fisher's least significance difference (LSD) test was then used to compare means of lesion lengths of isolates and F-values with $P < 0.05$ were considered significant. Isolations were then carried out by cutting 12 small wood pieces (~1 × 1 mm) along the lesions and transferring them to PDA-C in Petri dishes. The dishes with wood pieces were incubated at 24°C for 4 weeks with approximately 12 h of daylight and 12 h of darkness and frequently checked for fungal growth. Isolates of Diatrypaceae species were transferred to new PDA-C dishes. Three representatives

of each isolate per species were subjected to DNA extraction and sequencing of the partial β -tubulin gene, as described above, to confirm that reisolated species were the same as the ones used for inoculation.

Pathogenicity on mature trees. A total of 15 isolates, three isolates of each of the five species of Diatrypaceae, were plated onto PDA-C and incubated at 24°C for 2 weeks for use in the field pathogenicity test. Inoculations were made on shoots, from the previous year's growth, of field-grown apricots (cv. Bulida) and plums (cv. Pioneer). Noncolonized PDA plugs were used to inoculate control shoots. A total of 10 shoots (per host) were used for each isolate, and a 4-mm cork borer was used to wound the shoots before a colonized agar plug from a 2-week-old culture was placed in each wound, which was then covered with Parafilm. The trials were laid out in an incomplete block design. After 5 months, inoculated shoots were removed from the trees and taken to the laboratory for isolations. Before isolation, the shoots were split longitudinally through the inoculation point and lesion length was measured from both directions of the inoculation point. Reisolations and statistical analyses were carried out as described above.

Results

Symptoms in orchards. A total of 380 wood samples were collected from different stone fruit orchards in the Western Cape and Limpopo Provinces, South Africa. The most common external symptoms observed during sampling included branch dieback, cankers associated with pruning wounds and often with gum exudation, as well as tree death (Fig. 1). Internal symptoms included watery necrosis, roundish brown discoloration of wood (either at the center or the margin of the branches), and wedge-shaped necrosis when samples were cross-sectioned (Fig. 1, Table 2).

Fungal isolates. A total of 46 strains of Diatrypaceae were obtained. Most of the isolates were obtained from apricot (65%), followed by plum (24%), sweet cherry (9%), and almond (2%). Strains of Diatrypaceae obtained in this study were assigned to five species, namely *Cryptovalsa ampelina*, *E. lata*, *Eutypa cremea* Moyo, Halleen, L. Mostert,

Table 2. External and internal disease symptoms associated with Diatrypaceae species, frequency and number of fungal isolates obtained from apricot, plum, almonds, and sweet cherry in South Africa

Host (bold), Fungus	Number of isolates	Location (Province) ^z	Symptoms	
			External	Internal
<i>Prunus armeniaca</i>				
<i>Eutypa lata</i>	15	Bonnievale, Calitzdorp, Montagu, Robertson, Stellenbosch (WC)	Branch canker associated with pruning wound	Wedge-shaped necrosis, black/brown discoloration
<i>Eutypella citricola</i>	4	Bonnievale, Calitzdorp (WC), Mookgophong (LP)	Branch dieback with gummosis	Wedge-shaped necrosis
<i>Cryptovalsa ampelina</i>	4	Bonnievale, Robertson (WC)	Branch dieback, canker and gummosis	Dark-brown discoloration
<i>Eutypella microtheca</i>	2	Calitzdorp, Robertson (WC)	Branch dieback and gummosis	Black/brown discoloration associated with wound
<i>Eutypa cremea</i>	5	Bonnievale, Montagu (WC)	Branch dieback and canker	Black/brown discoloration associated with wound
<i>Prunus salicina</i>				
<i>Eutypa lata</i>	7	Franschhoek, Stellenbosch (WC)	Branch dieback and cankers	Wedge-shaped necrosis, brown discoloration from pruning wound
<i>Eutypella citricola</i>	1	Bonnievale (WC)	Branch dieback with gummosis	Wedge-shaped necrosis
<i>Cryptovalsa ampelina</i>	1	Paarl (WC)	Branch dieback	Brown discoloration of wood
<i>Eutypella microtheca</i>	1	Mookgophong (LP)	Canker with gummosis	Brown necrosis associated with canker
<i>Eutypa cremea</i>	1	Stellenbosch (WC)	Canker	Brown discoloration
<i>Prunus dulcis</i>				
<i>Eutypa lata</i>	1	Robertson (WC)	Canker	Brown/black discoloration
<i>Prunus avium</i>				
<i>Eutypa lata</i>	3	Ceres (WC)	Canker	Brown discoloration extending from wound
<i>Cryptovalsa ampelina</i>	1	Ceres (WC)	Perithecia	...

^z WC: Western Cape Province; LP: Limpopo Province.

Eutypella citricola Speg., and *Eutypella microtheca* Trouillas, W.M. Pitt & Gubler. Maximum likelihood analysis of the individual ITS and β -tubulin sequences, as well as of the combined data set, generated trees with similar topology with regards to the clades/subclades representing species isolated in this study. The ML tree supporting species identification is presented in Figure 2. The most abundant species obtained by isolating from stone fruit trees was *E. lata* with 26 isolates, followed by *C. ampelina* with seven isolates. *E. citricola* and *E. cremea* were isolated five times each; whereas three isolates of *E. microtheca* were obtained (Table 2). Although apricot and plum trees were colonized by the same species of Diatrypaceae, the number of isolates collected from these hosts was very different, constituting 30 and 11 isolates, respectively. All isolates were obtained from internal wood necrosis of living branches of stone fruit trees, except for one *C. ampelina* isolate, which was isolated from perithecia on dead wood of sweet cherry collected from the orchard floor. The origin, number of isolates, and the different symptoms from which fungal species were isolated are presented in Table 2.

Pathogenicity. The preliminary laboratory test showed that all species, except for *E. citricola* on plum, produced lesions on apricot and plum, which were significantly longer than the controls. Species produced longer lesions on apricot compared with plum (Table 3).

The lesions produced on apricot often extended the full length of the shoots or at least nearly so with little differences between the species. On apricot, lesions caused by *E. cremea* and *E. lata* were longer than those caused by *C. ampelina*, *E. citricola*, and *E. microtheca*, but *E. lata* and *E. microtheca* produced the longest lesions on plum.

Table 3. Mean lesion lengths in detached apricot and plum shoots, 2 weeks after inoculation with different Diatrypaceae species, and mean reisolation percentages of these species from observed lesions

Fungal species	Apricot		Plum	
	Mean lesion length (mm) ^z	Reisolation percentage	Mean lesion length (mm) ^z	Reisolation percentage
<i>Eutypa lata</i>	120.0 a	21.0	108.9 a	29.5
<i>Eutypa cremea</i>	119.2 a	42.0	86.9 b	12.5
<i>Eutypella microtheca</i>	111.6 ab	62.5	110.2 a	70.5
<i>Cryptovalsa ampelina</i>	109.6 ab	20.5	44.3 c	20.5
<i>Eutypella citricola</i>	100.0 b	50.0	33.2 cd	8.0
Uncolonized agar plug	25.6 c	...	19.2 d	...
LSD ($P < 0.05$)	16.3	...	18.0	...

^z Means followed by the same letter in each column are not significantly different ($P < 0.05$).

Fig. 2. The maximum likelihood phylogenetic tree of species in the Diatrypaceae based on ITS and β -tubulin sequence data. Bootstrap support values were calculated from 1,000 replicates and bootstrap support of $<60\%$ are not shown. *Hypoxylon fragiforme* served as an outgroup. Isolates obtained in this study are in bold.

All species were reisolated from the margins of necrotic tissues of apricots and plum with *E. microtheca* having the highest recovery percentages from both hosts (Table 3). No fungi were isolated from control shoots.

On field-grown apricot and plum trees inoculated with isolates of Diatrypaceae species, lesions were produced within 5 months, and mean comparison tests showed that all isolates, except for *E. microtheca* STEU 6086 on apricot, produced lesions significantly longer than the controls (Table 4). Lesions produced by isolates of different species on apricot and plum were brown-red in color (Figs. 3 and 4), which is characteristic for symptoms of Eutypa dieback of apricot (Carter and Moller 1977). The results showed that apricot was more susceptible to the inoculated diatrypaceous species than plum (Table 4), confirming the results observed in the detached shoot trial. Mean lesion lengths ranged from 28.9 mm for *E. microtheca* STEU 6086 to 66.0 mm for *E. lata* STEU 6082 on apricot and 16.3 mm for *E. lata* STEU 6084 to 35.5 mm for *E. microtheca* STEU 8058 on plum. Significant differences in lesion lengths were found among species as

well as within species both in apricot ($F = 5.87$, $P < 0.0001$) and plum wood ($F = 5.92$, $P < 0.0001$). Not all isolates, however, performed similarly in apricot and plum wood. For example, *E. lata* STEU 6084 was among the four most virulent isolates on apricot but was the least virulent isolate on plum; and *E. microtheca* STEU 6086 was the least virulent isolate on apricot where it produced lesions similar to that of the control, but was the second most virulent isolate on plum. All isolates were reisolated at frequencies of 50 to 100%, while no Diatrypaceae species were isolated from the control shoots (Table 4).

Discussion

This study reports for the first time the diversity of Diatrypaceae species associated with stone fruit trees in South Africa. These species were associated with symptoms including wedge-shaped necrosis, cankers, and dieback, which were also observed on grapevine affected by dieback worldwide (Díaz et al. 2011; Luque et al. 2012; Moyo et al. 2018; Trouillas et al. 2010). The discovery of

Table 4. Mean lesion lengths in shoots of apricot and plum trees, five months after artificial inoculation with isolates of Diatrypaceae species, and percentage reisolation of the individual isolates.

Species	STEU number	Origin of isolate ^y	Apricot		Plum	
			Mean lesion length (mm) ^z	Reisolation percentage	Mean lesion length (mm) ^z	Reisolation percentage
<i>Cryptovalsa ampelina</i>	5947	Paarl, WC	53.4 a-d	100	21.1 c-e	60
<i>Cryptovalsa ampelina</i>	6087	Robertson, WC	39.8 ef	90	17.5 de	70
<i>Cryptovalsa ampelina</i>	8056	Bonnievale, WC	47.7 b-e	90	24.5 b-d	60
<i>Eutypa lata</i>	6081	Montagu, WC	56.5 a-c	80	20.4 c-e	100
<i>Eutypa lata</i>	6082	Robertson, WC	66.0 a	90	29.2 ab	90
<i>Eutypa lata</i>	6084	Franschhoek, WC	58.6 ab	90	16.3 e	100
<i>Eutypa cremea</i>	5832	Stellenbosch, WC	49.3 b-e	80	20.3 c-e	100
<i>Eutypa cremea</i>	6085	Montagu, WC	49.3 b-e	90	20.1 c-e	90
<i>Eutypa cremea</i>	8057	Bonnievale, WC	45.4 b-e	100	18.8 de	80
<i>Eutypella citricola</i>	5948	Mookgophong, LP	37.0 ef	70	22.3 b-e	80
<i>Eutypella citricola</i>	8054	Bonnievale, WC	43.3 c-e	70	17.8 de	80
<i>Eutypella citricola</i>	8055	Bonnievale, WC	40.5 d-f	50	28.0 a-c	70
<i>Eutypella microtheca</i>	6086	Robertson, WC	28.9 fg	70	32.5 a	100
<i>Eutypella microtheca</i>	5949	Mookgophong, LP	40.6 d-f	100	18.3 de	80
<i>Eutypella microtheca</i>	8058	Calitzdorp, WC	63.6 a	100	35.5 a	60
Uncolonized PDA plug	...	...	18.1 g	0	4.8 f	0
LSD ($P < 0.05$)			13.2		8.0	

^y WC: Western Cape Province; LP: Limpopo Province.

^z Means followed by the same letter in the same column are not significantly different ($P < 0.05$).

Fig. 3. Necroses in shoots of field-grown apricot trees of the cv. Bulida caused by Diatrypaceae species 5 months after inoculation. A, *Eutypa cremea*. B, *Eutypella citricola*. C, *Cryptovalsa ampelina*. D, *Eutypa lata*. E, *Eutypella microtheca*. F, Control.

the same species in wood of grapevine (Moyo et al. 2018) and stone fruit trees (this study) provides circumstantial evidence that their inoculum travels between these hosts in South Africa. However, it is likely that not only stone fruit trees and grapevine provide inoculum for each other, but also several tree hosts occurring in close proximity can serve as inoculum sources for stone fruit trees and grapevine and vice versa. For instance, three Diatrypaceae species found in this study, namely *E. lata*, *E. citricola*, and *E. microtheca* were also found in persimmon orchards in South Africa (Moyo et al. 2016). Persimmon and stone fruit orchards are often planted adjacent to vineyards as well as to each other in South Africa. Moreover, examinations of diseased stone fruit trees during our surveys did not yield any perithecia with the exception of *C. ampelina* on sweet cherry. In contrast, perithecia of the five Diatrypaceae species, isolated in this study, are often found in vineyards in the Western Cape (Moyo et al. 2018). The lack of perithecia in stone fruit orchards may be an indication that inoculum of these pathogens did not primarily originate from the stone fruit orchards, but from outside sources, or it may be due to the less intensive search done for these fruiting bodies in this study.

E. lata is a well-known pathogen of apricot and other stone fruit trees in different countries including South Africa (Carter 1957; Matthee et al. 1974; Munkvold and Marois 1994; Rumbos 1997). Of the species found in this study, *E. lata* was the most encountered and was found to occur on all *Prunus* species, which yielded Diatrypaceae isolates. This fungus is also the most widespread Diatrypaceae species globally and its dominance in this study is, therefore, not surprising. This study also revealed the presence of *C. ampelina*, *E. cremea*, *E. citricola*, and *E. microtheca* in wood of stone fruit trees, of which the latter three are reported for the first time on stone fruits worldwide. *C. ampelina* has been reported on apricot (Trouillas et al. 2010) and sweet cherry trees in California (Trouillas et al. 2012).

The present study showed that apricot and plum trees harbored more Diatrypaceae species (five species each) than sweet cherry and almond trees, which yielded two and one species, respectively. Although Diatrypaceae species were isolated from almonds and cherry as well, the diversity was limited and pathogenicity were not conducted with these hosts in the present study. The differences in the diversity of Diatrypaceae species colonizing the different *Prunus* species could probably be attributed to several factors. For instance, sweet cherries are cultivated in a climatically different region than most other fruit types because of these trees' cold requirement (Küden et al. 1997) and probably because of the fewer samples collected for sweet cherry and almond compared with plum and apricot. Host defense mechanisms can also play a role, with some hosts

possessing biochemical characteristics that limit extensive colonization by the fungal species. It would seem that peach and nectarine are not susceptible to attack by Diatrypaceae species and this is shown by the failure to detect these species from these host plants in this study and the lack of reports of natural infection of these two hosts in literature. *E. lata*, however, has been shown to be pathogenic to peach by artificial inoculation (English and Davis 1978; Matthee et al. 1974; Munkvold 2001).

Results of pathogenicity tests in the present study showed that all Diatrypaceae isolates tested were pathogenic causing brown-red lesions, which are typical of Eutypa dieback, on apricot and plum shoots. This is the first demonstration of pathogenicity of *C. ampelina*, *E. cremea*, *E. citricola*, and *E. microtheca* on stone fruit trees. However, their aggressiveness to plum was less compared with apricot. Based on the number of isolates obtained and the pathogenicity tests in this study, it seems that at least in South Africa, apricot is more susceptible to Diatrypaceae infection than plum. Interestingly, it was also noted that apricot was more sensitive to wounding than plum. Results also showed that *E. lata* was the most pathogenic species on apricot, which indicates that this species is still an important pathogen of apricot in South Africa. In this study, *C. ampelina*, *E. cremea*, *E. citricola*, and *E. microtheca* were also pathogenic to apricot, with some isolates of these species producing lesions even equivalent to those of *E. lata*. Differences in virulence among isolates of *C. ampelina* and *E. microtheca* on apricots were also observed. Differences in virulence among strains of Diatrypaceae species are, however, not surprising given that such differences have been documented for species including *E. lata* and *C. ampelina* on grapevine (Péros et al. 1997; Trouillas and Gubler 2010). Lesions observed in the field trials were shorter than those observed in the detached shoot assays. The longer lesions observed during the detached shoot assays could probably be attributed to detached shoots having less defense mechanisms that are active enough to prevent or reduce infection and the warm, humid incubation conditions in the laboratory.

No cankers were observed during the pathogenicity trials and this could be explained by the short incubation period of the pathogenicity tests. Further experiments with longer incubation periods are therefore required to confirm the potential of the tested species to cause cankers as well as foliar symptoms. Nevertheless, despite the fact that dieback symptoms were not reproduced in the present study, the ability of Diatrypaceae species to cause dieback and cankers is strongly assumed as a result of orchard observations and the isolation of these species from necrotic areas of active cankers of stone fruit trees. The results of this study are a good foundation for future

Fig. 4. Necroses in shoots of field-grown plum trees of the cv. Pioneer caused by Diatrypaceae species 5 months after inoculation. **A**, *Eutypa cremea*. **B**, *Eutypella citricola*. **C**, *Cryptovalsa ampelina*. **D**, *Eutypa lata*. **E**, *Eutypella microtheca*. **F**, Control.

research aimed at managing infections caused by Diatrypaceae species on stone fruit trees in South Africa, as well as providing the grapevine and fruit industries with new knowledge regarding the host ranges of Diatrypaceae species and integrated management strategies to control these species in areas of diverse fruit production.

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