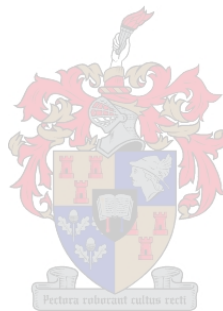# OCCURRENCE OF CANKER AND WOOD ROT PATHOGENS ON STONE FRUIT PROPAGATION MATERIAL AND NURSERY STONE FRUIT TREES

by

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DECLARATION

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SUMMARY

The phytosanitary status of stone fruit propagation material and nursery trees in South Africa are not known. Canker and wood rot pathogens can be present in visibly clean material. Due to stress and other improper cultural practices, symptoms will be expressed and cankers, dieback of parts of the tree and possible death of the trees can be seen. Therefore, the aim of this study was to identify the fungal canker and wood rot pathogens present in propagation material and nursery stone fruit trees.

Green scion shoots were collected from three plum and one nectarine cultivars and dormant scion shoots were collected from three plum cultivars. The rootstock cultivars included three plum and one nectarine cultivar for the dormant rootstock shoots and three plum and two nectarine cultivars for the ungrafted rooted, rootstock plants. Nursery trees, made with the same combinations of scion and rootstock cultivars were also sampled. All the plant material were surface sterilised and isolations were made from the different plant material types. The fungal cultures were identified to species level with DNA sequencing and phylogenetic analyses of either ITS, β-tubulin, EF1α and histone gene regions.

From the green scion shoots, low levels of bud infection were observed. In total 0.4% buds were infected with canker pathogens with one bud infected with "*Cylindrocarpon*"-like fungi. The most abundant species isolated were *Coniochaeta prunicola* followed by *Biscogniauxia* sp. Buds from dormant scion shoots had a higher total infection of 1.2% with canker pathogens. *Truncatella angustata* followed by *Didymella pomorum* were the species isolated the most.

Dormant rootstock shoots had 6.2% of shoots infected with canker pathogens. The pathogens that were isolated most often include *Cytospora leucostoma*, *Diplodia seriata* and *Didymella pomorum*. From the total ungrafted, rooted rootstock plants (378), 10.6% were infected with canker and wood rot pathogens and 6.4% were infected with "*Cylindrocarpon*"-like fungi. The nectarine rootstock which had a low level of infection (1.3%) can be explained by the fact that these plants were made from seeds. *Cadophora* and *Dactylonectria* species were the most abundant. Rootstock plants were mainly infected at the crown, but also below the pruning wound at the tip of the main shoot.

Out of 1080 nursery trees that were sampled, 235 trees (21.8%) had infection with canker or wood rot pathogens and 255 trees (23.6%) had infection with "*Cylindrocarpon*"-like fungi. *Cadophora luteo-olivacea*, *Diplodia seriata* and *Truncatella angustata* were the most abundant canker species isolated from the nursery trees. There were clear differences between the infection percentages of the trees being propagated by using hardwood rootstock cuttings in comparison with the seedling rootstock trees.

The propagation material investigated had infections with canker pathogens. Buds from scion shoots had very low levels of infection. Ungrafted, rooted rootstock plants had

higher levels of infection than the dormant rootstock shoots, although there were already pathogens that occur inside the dormant rootstock shoots before it was planted in the field. Infections were more often at the crown area of the rootstock plants and accompanied with dark brown streaking originating from the base. It is evident that rootstock cuttings that are pushed into the soil have an open wound that can easily be infected.

Fifty-five fungal species associated with canker or wood rot were isolated in this study. Nineteen have been reported on stone fruit trees in South Africa and 26 are first reports on stone fruit trees in South Africa, which include species of the genera *Biscogniauxia*, *Cadophora*, *Coniochaeta*, *Coprinellus*, *Cytospora*, *Diaporthe*, *Didymella*, *Dothiorella*, *Eutypa*, *Lasiodiplodia*, *Neopestalotiopsis*, *Paraphaeosphaeria*, *Paraphoma*, *Pleurostoma*, *Truncatella* and *Valsa*. Four of these species have been reported on stone fruit trees in other countries, thus, 22 species have been reported for the first time on stone fruit trees worldwide. Ten putative new species were found which include species of *Peniophora*, *Cadophora*, *Coniochaeta*, *Eutypella*, *Cytospora* and *Biscogniauxia*, however, these species needs to be described. A pathogenicity trial done on field grown plum trees confirmed the pathogenic status of 38 of the canker and wood rot species to be pathogenic to plum trees four months after inoculation. None of the 14 "*Cylindrocarpon*"-like species have been reported on stone fruit trees in South Africa. Only *Ilyonectria robusta* have been reported on stone fruit in Canada. The pathogen status and relevance of the "*Cylindrocarpon*"-like fungi needs to be determined with pathogenicity trials.

This study has found that seemingly healthy, certified nursery trees with latent canker and wood rot pathogens and "*Cylindrocarpon*"-like fungi present inside the plant tissue, are distributed to producers. This fungal infection could have occurred from the propagation process with infected scion and rootstock material or from aerial inoculum present when wounds were made. These findings will aid to identify areas where management practices can be implemented to improve nursery plant health.

OPSOMMING

Die fitosanitêre status van steenvrug entmateriaal en kwekery bome is nie bekend in Suid-Afrika nie. Kanker en houtverrottingspatogene kan voorkom in ooglopende skoon materiaal. As gevolg van stres toestande en ander verkeerde landbou praktyke, kan simptome soos kankers, terugsterwing van gedeeltes van die boom en die moontlike sterwing van die hele boom waargemeem word. Die doel van die studie was dus om die kanker en houtverrottings patogene te identifiseer wat voorkom in steenvrug entmateriaal en kwekery bome.

Groen bo-stok lote was ingesamel van drie pruim kultivars en een nektarien kultivar en dormante bo-stok lote was ingesamel van drie pruim kultivars. Die onderstok kultivars sluit drie pruim kultivars en een nektarien kultivar in vir die dormante onderstok lote en drie pruim en twee nektarien kultivars vir die ongeënte, gewortelde onderstok plante. Kwekery bome, wat gemaak is met dieselfde bo-stok en onderstok kultivars, was ook ingesamel. Al die plantmateriaal was oppervlak gesteriliseer en isolasies was gemaak van die verskillende plantmateriaal tipes. Die swam kulture was identifiseer tot op spesie vlak met DNS volgorde bepaling en filogenetiese analise van die ITS, β-tubulin, EF1α en histone geen areas.

Lae infeksie vlakke was gesien vir die groen bo-stok lote. In totaal was 0.4% van die ogies infekteer met kanker patogene en een ogie infekteer met `n "*Cylindrocarpon*"-assossieerde swam. Die spesies wat die meeste isoleer was, was *Coniochaeta prunicola* gevolg deur *Biscogniauxia* sp. Ogies van die dormante bostok lote het `n hoër totale infeksie gehad van 1.2% met kanker patogene. *Truncatella angustata* gevolg deur *Didymella pomorum* was die spesies wat die meeste isoleer was.

Die dormante onderstok lote het 6.2% infeksie met kanker patogene gehad. Die patogene wat die meeste isoleer was, sluit *Cytospora leucostoma*, *Diplodia seriata* en *Didymella pomorum* in. Van die totale ongeënte, gewortelde onderstok plante (378), was 10.6% infekteer met kanker en houtverrottings swamme en 6.4% infekteer met "*Cylindrocarpon*"-assossieerde swamme. Die nektarien onderstok met die lae infeksie (1.3%), kan verduidelik word deurdat hierdie plante saailinge is. *Cadophora* spesies en *Dactylonectria* spesies was die meeste gevind. Die onderstok plante was hoofsaaklik infekteer by die kroon, maar ook onder die snoeiwond by die tip van die hoof loot.

Uit die 1080 kwekery bome wat ingesamel is, was 235 bome (21.8%) infekteer met kanker en houtverrottings patogene en 255 bome (23.6%) infekteer met "*Cylindrocarpon*"-assossieerde swamme. *Cadophora luteo-olivacea*, *Diplodia seriata* en *Truncatella angustata* was die spesies wat die meeste isoleer was van die kanker swamme vanaf die kwekery bome. Daar was duidelike verskille in die infeksie persentasies tussen die bome gemaak van hardehout onderstok steggies in vergelyking met saailing onderstok plante.

Die entmateriaal wat ondersoek was, het infeksies gehad met kanker patogene. Ogies van die bostok lote het baie lae infeksie gehad. Ongeënte, gewortelde onderstok plante het

hoër infeksie gehad as die dormante onderstok lote, alhoewel daar alreeds patogene in die dormante onderstok lote was nog voor dit in die veld uitgeplant was. Infeksie was meer gereeld by die kroon gedeelte van die onderstok plante tesame met donker bruin verkleuring vanaf die basis. Dit is duidelik dat onderstok steggies wat in die grond gedruk word `n oop wond het wat maklik infekteer kan word.

Vyf-en-vyftig spesies wat assosieer word met kanker en houtverrotting was isoleer in hierdie studie. Negentien is reeds rapporteer op steenvrugte in Suid-Afrika en 26 is eerste rapporterings op steenvrugte in Suid-Afrika, wat spesies van die genera *Biscogniauxia*, *Cadophora*, *Coniochaeta*, *Coprinellus*, *Cytospora*, *Diaporthe*, *Didymella*, *Dothiorella*, *Eutypa*, *Lasiodiplodia*, *Neopestalotiopsis*, *Paraphaeosphaeria*, *Paraphoma*, *Pleurostoma*, *Truncatella* en *Valsa* in sluit. Vier van hierdie spesies was al rapporteer op steenvrugte in ander lande, dus 22 spesies was vir die eerste keer op steenvrugte wêreldwyd rapporteer. Tien vermeende nuwe spesies was gevind wat *Peniophora*, *Cadophora*, *Coniochaeta*, *Eutypella*, *Cytospora* en *Biscogniauxia* in sluit, maar hierdie spesies moet nog beskryf word. `n Patogenesiteitstoets wat gedoen was op pruim bome in die veld het die patogeen status van 38 van die kanker en houtverrottings spesies bevestig vier maande na inokulasie. Geen van die "*Cylindrocarpon*"-assossieerde swamme was al rapporteer op steenvrugte in Suid-Afrika nie. Slegs *Ilyonectria robusta* was rapporteer op steenvrugte in Canada. Die patogeen status en relevansie van die "*Cylindrocarpon*"-assossieerde swamme moet nog bepaal word met patogenesiteitstoetse.

Die studie het gevind dat oënskynlik gesonde, gesertifiseerde kwekery bome met latente kanker en houtverrotting patogene en "*Cylindrocarpon*"-assossieerde swamme teenwoordig binne die plantweefsel, versprei word aan produsente. Hierdie swam infeksie kon ontstaan vanaf die ent prosses met infekteerde bostok en onderstok materiaal of van luggedraagde inokulum teenwoordig wanneer wonde gemaak was. Hierdie bevindinge sal toevoeg om areas te identifiseer waar bestuurspraktyke implimenteer kan word om kwekery plante se gesondheid te verbeter.

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CONTENTS

CHAPTER 1

A review of canker and wood rot of stone fruit trees

INTRODUCTION

Canker and wood rot diseases are of great concern to commercial farmers as it can result in extensive losses in yield if the diseases are not managed (Matthee and Thomas, 1977a; Slippers and Wingfield, 2007; Gramaje et al., 2012). Canker and wood rot pathogens can occur in symptomless trees as endophytes (Smit et al., 1996) and when the trees are exposed to stress symptoms, the disease start developing (Pusey, 1989). The infection of young trees lead to replacement and re-establishing of new trees and even if the infected trees survive, it will still result in lower yield. As a result of the increase in establishment costs (Hortgro, 2017), there is a high demand from producers to plant disease free trees, to avoid any economic losses of young trees.

Canker and wood rot are important diseases of stone fruit causing dieback and potentially also tree mortality. External disease symptoms include cankers on twigs, branches and main trunks, dieback, blight, gummosis and in severe cases death of the plant (Slippers et al., 2007; Van Niekerk et al., 2011; Gramaje et al., 2012; Mostert et al., 2016a). Internal symptoms can be described as vascular streaking with a brown to black colour and when transversely cut wedge- or V-shaped necrosis, spots or circular discolouration can be observed (Matthee and Thomas, 1977a; Damm et al., 2007a; Mostert et al., 2016a). The main fungal groups of pathogens associated with cankers and wood rot include Botryosphaeriaceae, Calosphaeriaceae, Tympanidaceae, Coniochaetaceae, Diaporthaceae, Diatrypaceae, Didymosphaeriaceae, Phaeomoniellaceae, Togniniaceae, Valsaceae and Basidiomycetes (Ogawa et al., 1995; Gramaje et al., 2012; Mostert et al., 2016a; Havenga, 2017). Cankers on stone fruit trees can also be due to bacteria. *Pseudomonas syringae* pv. *syringae* and *Pseudomonas syringae* pv. *morsprunorum* are both associated with bacterial canker of stone fruit trees (Bultreys and Kaluzna, 2010; Stefani, 2010; Lópes et al., 2011). This review will, however, focus on fungal canker pathogens.

A recent study in South Africa showed that 65% of 480 certified nursery apple trees that were visually clean from any disease symptoms were infected with canker and/ or wood rot pathogens (Havenga, 2017). The same study also showed that similar pathogens were found in nursery trees as well as in propagation material (Havenga, 2017). The Deciduous Fruit Plant Improvement Scheme provides a list of all the pathogens and pests that are tested for to ensure that nursery pome and stone fruit trees are visually disease free (Van Rensburg, 1997). This list does not include important canker and wood rot pathogens. Apart from this visual

inspections of nursery trees, the scheme does not allow for the detection of internal symptoms or latent infections of canker or wood rot pathogens. The occurrence of canker and wood rot pathogens in nursery stone fruit trees is not known.

The current literature study will give an overview of the South African stone fruit industry as well as global stone fruit production. It will examine the pathogens associated with canker and wood rot of stone fruit trees, in South Africa as well as globally. Their epidemiology and symptoms will also be reviewed and possible control methods will be given for these diseases. The propagation process and the production of nursery trees will be discussed. Lastly, the aim and objectives of the current study will be provided.

STONE FRUIT INDUSTRY

Stone fruit is a term that is used to describe a collection of different fruit types, which include apricots (*Prunus armeniaca* L.), peaches (*Prunus persica* (L.) Batsch.), nectarines (*Prunus persica* var. *nucipersica* (Suckow) C.K. Schneid.), plums (*Prunus salicina* Lindl.) and cherries (*Prunus avium* (L.) L.). Most of the stone fruit types have originated in the East. Apricot and peach trees originated in China and plums originated in Europe and Japan (Ogawa *et al.*, 1995).

South African stone fruit production

Stone fruit is one of the most important fruit crops with over 17 600 hectares cultivated in South Africa. More than 320 000 tons was harvested in 2017 in South Africa, with a turnover of R2,7 Bn. Of the different stone fruit types, plums are of higher economic value due to higher export volumes (Hortgro, 2017).

Stone fruit consist of temperate fruit types and are grown in regions with not too high or too low temperatures. Stone fruit is cultivated in all of the provinces of South Africa. The largest portion of South Africa's stone fruit is grown in the western areas of South Africa, and consists of the Klein Karoo, Langkloof, Ceres, EGVV (Elgin/Grabouw/Villiersdorp/Vyeboom) and the Bergriver production area. Stone fruit are also farmed in the Northern Province and Mpumalanga, but to a smaller extent. The Western Cape with a Mediterranean-type climate, is characterised by wet and cold winters and hot and dry summers. These climatic conditions are suitable for stone fruit cultivation (Taylor and Gush, 2007). Cool temperatures are required for good fruit colour and to induce dormancy. Stone fruit trees can be grown and will do good on any soil type, but it is important to have well drained soil and the site where trees are planted should also be in full sunlight (Lord and Ouellette, 2013). Moisture caused by rain and high humidity during the growing season can promote diseases which can cause a decrease in the yields (Taylor and Gush, 2007).

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The stone fruit types vary in regards to the volumes exported and delivered to the local markets. The latter consisting of market sales and direct sales to supermarkets, processed and dried fruit. During 2017, 6% of apricots and 8% of peaches and nectarines were exported, while 35% of cherries and 74% of plums were exported (Table 1) (Hortgro, 2017). Table 2 shows the largest export markets where stone fruit crops were exported to in 2017. The Middle East, United Kingdom and Europe were the three markets where stone fruit were exported to most. Plums, which is the highest exported crop, was mostly exported to Europe (Table 2) (Hortgro, 2017).

Global stone fruit production

Stone fruit is not only of economic importance for South Africa, but also worldwide. According to recent data by FAOSTAT (2017), the top apricot producing countries are Turkey, Uzbekistan and Algeria, the top peach and nectarine producing countries are China, Italy and United States of America and the top plum producing countries are China, Serbia and Romania, all in descending order. South Africa falls into the southern hemisphere of production regions. Chile is the largest southern hemisphere producer of deciduous fruit and together with South Africa the main exporters of deciduous fruit in the southern hemisphere with 71% being exported by Chile and South Africa collectively (Hortgro, 2017). In the southern hemisphere, South Africa is the largest apricot exporter and the second largest exporter of peaches, nectarines and plums after Chile (Hortgro, 2017). Worldwide, South Africa is ranked lower compared to other production regions: 24th for apricot production, 16th for peach and nectarine production, 21st for plum production and 62nd for cherries (Hortgro, 2017).

CANKER AND WOOD ROT ON STONE FRUIT TREES

A wide diversity of Ascomycetes species (76) have been identified as canker or dieback causing pathogens on *Prunus* spp. worldwide (Addendum A, Table 1). Of these, 51 species have been found on stone fruit trees in South Africa (Doidge, 1950; Gorter, 1977; Smit *et al.*, 1996; Crous *et al.*, 2000; Slippers *et al.*, 2007; Damm *et al.*, 2007 a,b; Damm *et al.*, 2008 a,b,c; Damm *et al.,* 2010; Santos *et al.*, 2017; Jami *et al.*, 2017; Moyo *et al.*, 2018; Spies *et al.*, 2018). In total, 18 Basidiomycetes fungi have been associated with wood rot on *Prunus* spp. (Addendum A, Table 2). The host diversity from which canker and wood rot pathogens were isolated from include a wide range of *Prunus* spp. (Addendum A, Table 1 and 2). Among others, *Prunus armeniaca*, *Prunus avium*, *Prunus domestica* L., *Prunus dulcis* (Mill.) D.A. Webb., *Prunus persica*, *Prunus persica* var. *nucipersica* and *Prunus salicina* were the most observed *Prunus* host species to canker and wood rot pathogens.

Canker and wood rot pathogens found on stone fruit trees in South Africa

The Botryosphaeriaceae are considered as one of the most common pathogen groups that causes dieback and cankers of woody plants, especially stone fruit trees (Damm *et al.*, 2007a; Slippers *et al.*, 2007). Damm *et al.* (2007a) conducted a study on the Botryosphaeriaceae on stone fruit trees in South Africa and identified *Diplodia africana* (Tuck.) Matzer, H. Mayrhofer & Rambold, *Diplodia mutila* (Fr.) Mont., *Diplodia pinea* (Desm.) J. Kickx f., *Diplodia seriata* De Not., *Neofusicoccum australe* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, *Neofusicoccum vitifusiforme* (Van Niekerk & Crous) Crous, Slippers & A.J.L. Phillips, *Dothiorella viticola* A.J.L. Phillips & J. Luque and *Lasiodiplodia plurivora* Damm & Crous. *Aplosporella prunicola* Damm & Crous was also identified on stone fruit trees in South Africa by Damm *et al.* (2007b). In the studies by Damm *et al.* (2007a) as well as Slippers *et al.* (2007), *D. seriata* was the most dominant species isolated from stone fruit trees in South Africa. All the species in the study by Damm *et al.* (2007a) caused lesions in a detached shoot assay on nectarine or plum shoots, except *Dot. viticola*. *Botryosphaeria dothidea* (Moug.) Ces. & De Not. is known to cause fungal gummosis on peach trees (Ogawa *et al.*, 1995) and was reported on stone fruit trees in South Africa by Crous *et al.* (2000).

The first report of species of the Calosphaeriaceae on stone fruit in South Africa were presented in a study done by Damm *et al.* (2008a), where *Jattaea prunicola* Damm & Crous and *Jattaea mookgoponga* Damm & Crous were described on plum and nectarine trees, respectively. *Calosphaeria africana* Damm & Crous were also isolated from apricot by Damm *et al.* (2008a). The first report of the genus *Coniochaeta* in South Africa was done by Damm *et al.* (2010). The same study reported *Coniochaeta velutina* (Fuckel) Cooke for the first time on stone fruit trees, however, *Con. velutina* did not show to be pathogenic to apricot, peach or plum shoots. *Coniochaeta prunicola* Damm & Crous and *Coniochaeta africana* Damm & Crous are two new species isolated from necrotic wood of stone fruit trees and formed significant lesions on apricot and peach shoots, respectively (Damm *et al.,* 2010).

In the Diaporthaceae, *Diaporthe ambigua* Nitschke was identified to cause cankers on apple, pear and plum rootstocks in South Africa (Smit *et al.,* 1996). *Diaporthe amygdali* (Delacr.) Udayanga, Crous & K.D. Hyde (synonym *Phomopsis amygdali* (Delacr.) J.J. Tuset & M.T. Portilla) is known to cause constriction canker on stone fruit trees (Ogawa *et al.*, 1995). This species has been found on grapevines in South Africa (Mostert *et al.*, 2001; Van Niekerk *et al.*, 2005), but not from stone fruit trees.

Moyo *et al.* (2018) characterised Diatrypaceae species occurring on stone fruit trees in the Western Cape. *Eutypa lata* (Pers.) Tul. & C. Tul. was the most commonly found, but *Cryptovalsa ampelina* (Nitschke) Fuckel*, Eutypa cremea* Moyo, Halleen, L. Mostert*, Eutypella citricola* Speg. *and Eutypella microtheca* Trouillas, W.M. Pitt & Gubler were also identified (Moyo *et al.*, 2018). All the species tested were shown to be pathogenic to apricot and plum,

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except *Eutypella microtheca* which was not pathogenic to apricot (Moyo *et al.*, 2018). Symptoms were observed as red-brown necrotic lesions in the pathogenicity trial where wood on the trees from the previous year`s growth were used (Moyo *et al.*, 2018).

Didymosphaeria rubi-ulmifolii Ariyaw., Camporesi & K.D. Hyde (synonym *Paraconiothyrium brasiliense* Verkley) was isolated from nectarine and plum branches showing necrotic symptoms in the wood (Damm *et al.*, 2008c). *Didymosphaeria variabile* (Riccioni, Damm, Verkley & Crous) Ariyaw. & K.D. Hyde (synonym *Paraconiothyrium variabile* Riccioni, Damm, Verkley & Crous) was isolated from necrotic wood of plum trees in South Africa and *Pseudocamarosporium africanum* (Damm, Verkley & Crous) Crous (synonym *Paraconiothyrium africanum* Damm, Verkley & Crous) was isolated from peach trees in South Africa (Damm *et al*., 2008c).

Celerioriella dura (Damm & Crous) Crous *(synonym Phaeomoniella dura* Damm & Crous) was isolated from wood of plum trees with symptoms of necrotic lesions and was described as a new species together with *Celerioriella prunicola* (Damm & Crous) Crous (synonym *Phaeomoniella prunicola* Damm & Crous) also from necrotic wood of plum trees in South Africa (Damm *et al.*, 2010). *Neophaeomoniella zymoides* (Hyang B. Lee, J.Y. Park, Summerb. & H.S. Jung) Crous (synonym *Phaeomoniella zymoides* Hyang B. Lee, J.Y. Park, Summerb. & H.S. Jung) was first reported in South Africa and isolated from plum trees (Damm *et al.*, 2010). In a pathogenicity test on detached green shoots none of the *Phaeomoniella* species were pathogenic to plum shoots, although *Cel. dura* was pathogenic to apricot shoots and *Neop. zymoides* was pathogenic to peach shoots (Damm *et al.*, 2010). There were no significant differences shown between *Cel. prunicola* and the non-pathogen controls (Damm *et al.,* 2010). *Aequabiliella effusa* (Damm & Crous) Crous (synonym *Phaeomoniella effusa* Damm & Crous) and *Minutiella tardicola* (Damm & Crous) Crous (synonym *Phaeomoniella tardicola* Damm & Crous) were also isolated from stone fruit trees, but were not pathogenic to plum, apricot or peach (Damm *et al.*, 2010).

Fourteen *Phaeoacremonium* species were isolated from stone fruit trees showing symptoms of dieback (Damm *et al.,* 2008b). The *Phaeoacremonium* species include: *Phaeoacremonium africanum* (Damm, L. Mostert & Crous) Gramaje, L. Mostert & Crous*, Phaeoacremonium australiense* L. Mostert, Summerb. & Crous*, Phaeoacremonium fraxinopennsylvanicum* (T.E. Hinds) Gramaje, L. Mostert & Crous*, Phaeoacremonium fuscum* L. Mostert, Damm & Crous*, Phaeoacremonium griseo-olivaceum* (Damm, L. Mostert & Crous) Gramaje, L. Mostert & Crous*, Phaeoacremonium griseorubrum* L. Mostert, Summerb. & Crous, *Phaeoacremonium iranianum* L. Mostert, Gräfenhan, W. Gams & Crous*, Phaeoacremonium minimum* (Tul. & C. Tul.) Gramaje, L. Mostert & Crous*, Phaeoacremonium pallidum* Damm, L. Mostert & Crous*, Phaeoacremonium parasiticum* (Ajello, Georg & C.J.K. Wang) W. Gams, Crous & M.J. Wingf.*, Phaeoacremonium prunicola* L. Mostert, Damm &

Crous, *Phaeoacremonium scolyti* L. Mostert, Summerb. & Crous, *Phaeoacremonium subulatum* L. Mostert, Summerb. & Crous and *Phaeoacremonium viticola* J. Dupont. *Phaeoacremonium alvesii* L. Mostert, Summerb. & Crous was reported on peach trees in South Africa in a study done by Spies *et al.* (2018). In the pathogenicity test done on detached plum and apricot shoots, all the *Phaeoacremonium* species isolated by Damm *et al.* (2008b) were pathogenic to plum, with the exception of *P. fuscum* and *P. pallidum*. Only *P. parasiticum, P. iranianum, P. subulatum, P. griseorubrum* and *P. africanum* were shown to be pathogenic to apricot (Damm *et al.*, 2008b).

The genus *Collophora* was first described by Damm *et al.* (2010) and five species have been originally found on stone fruit trees in South Africa, however, the genus *Collophora* was renamed as *Collophorina* by Wijayawardene *et al*., (2017) . The species found on stone fruit include *Collophorina africana* (Damm & Crous) Damm & Crous on plum trees, *Collophora capensis* Damm & Crous (synonym *Collophorina africana*) on plum trees, *Collophorina paarla* (Damm & Crous) Damm & Crous on plum and peach trees, *Collophora pallida* Damm & Crous (synonym *Collophorina paarla*) on plum and peach trees and *Collophorina rubra* (Damm & Crous) Damm & Crous on plum, peach, almond and nectarine trees. The pathogenicity test done on detached apricot, peach and plum shoots showed that *Col. africana* and *Col. rubra* were pathogenic to apricot, *Col. paarla* was pathogenic to plum, *Col. pallida* was pathogenic to peach and plum and *Col. capensis* was not pathogenic to any of the stone fruit cultivars used (Damm *et al.,* 2010).

Leucostoma canker was reported as a disease of stone fruit trees with *Leucostoma persoonii* (Nitschke) Höhn. and *Valsaria insitiva* (Tode) Ces. & De Not. (synonym *Leucostoma cincta* (Fr.:Fr.) Höhn.) being the causal pathogens (Ogawa *et al.*, 1995). The genera names *Leucostoma*, *Leucocytospora*, *Valsa*, *Valsella* and *Valseutypella* have been synonymised with *Cytospora*, however, the current names will be used for clarification (Rossman *et al*., 2015). *Leucostoma persoonii* has been found on peach trees in South Africa (Adams *et al*., 2006).

The Basidiomycetes species involved in wood rotting of stone fruit trees include a complex of pathogens in other countries (Addendum A, Table 2). Only *Armillaria mellea* (Vahl) P. Kumm., *Chondrostereum purpureum* (Pers.) Pouzar (synonym *Stereum purpureum* Pers.), *Schizophyllum commune* Fr. and *Trametes versicolor* (L.) Lloyd have been identified from stone fruit trees in South Africa (Doidge, 1950; Gorter, 1977; Crous *et al.*, 2000). *Chondrostereum purpereum* causes silver leaf disease on stone fruit trees (Ogawa *et al.*, 1995).

Isolation data of stone fruit trees showing symptoms of dieback, cankers or wood rot processed by the Disease Clinic (Plant Pathology Department, Stellenbosch University) from 2007 up to 2018 showed the relevance of the different fungal taxa in South Africa. From 170 stone fruit trees, 17 different fungal taxa were isolated (Table 3). The most canker and wood

rot fungi were isolated from plum trees (Table 4) with species of the Botryosphaeriaceae being isolated the most, followed by *Leucostoma*, *Diaporthe* and *Phaeoacremonium* species.

Symptoms associated with cankers and wood rot on stone fruit trees

Canker pathogens have the ability to be latent inside the plant material and only reveal symptoms when the trees experience stress conditions (Slippers and Wingfield, 2007). Symptoms related to fungal canker pathogens in South Africa are more clearly visible in areas where high moisture are observed and overhead irrigation is applied, whereas in drier areas there are few symptoms visible (Van Zyl, 2011). Cankers appears on twigs, branches and main stems of stone fruit trees causing dieback of the parts of the tree and in severe cases, death of the whole tree (Slippers and Wingfield, 2007). External canker lesions appear to be sunken, getting elongated and narrower to the margins of the lesions with cracks around the edges (Smit *et al.*, 1996). Some pathogens causes initial symptoms that are visible as gum exudation at the point of infection (Van Zyl, 2011; Sessa *et al.*, 2016).

The internal symptoms associated with fungal canker pathogen infection can be seen as brown to black vascular discolouration when the piece of infected wood are cut open in length (Gramaje *et al.*, 2012; Sessa *et al.*, 2016). In the cross section the symptoms appear as spots or circular discolouration in the xylem tissue (Gramaje *et al.*, 2012; Mostert *et al.*, 2016a). Most of the canker pathogens on stone fruit trees are isolated from wood showing internal symptoms that appear as wedge-shaped or V-shaped necrosis (Damm *et al.*, 2008a,b,c; Damm *et al.*, 2010; Gramaje *et al.*, 2012).

Basidiomycetes can colonize inside the vascular system, causing the wood to rot which block the vascular system and results in the dieback of the plant part above the blockage (Matthee and Thomas, 1977a; Takemoto *et al.*, 2010). Basidiomycetes can cause two types of wood decay on stone fruit trees known as white and brown rot (Ogawa *et al.*, 1995). Internal symptoms caused by wood rot fungi can be seen as discolouration in the wood, with white rot being more soft and whiter in colour in comparison to brown rot being brown and more brittle (Ogawa *et al.*, 1995). The external symptoms can be more spread out into a sunken lesion with brown discolouration and sometimes the appearance of papery bark and cracks (Matthee and Thomas, 1977a).

Severe spreading of the infection can lead to dieback of entire branches which will result in poor performance and production of stone fruit trees (Matthee and Thomas, 1977a). When canker and wood rot symptoms first appear in younger stone fruit trees, the whole tree is more rapidly killed compared to older trees, which are more resistant to infections and will only be killed over an extended time (Smit *et al.*, 1996).

Epidemiology of canker and wood rot pathogens

One of the largest sources of inoculum for the spreading of spores to cause infections are dead wood and branches in the orchards or in close proximity (Pusey, 1989). Varying between different fungal species of the Ascomycetes, conidia or ascospores can be released from respectively pycnidia or perithecia. These fruiting structures are found embedded on the surface of dead wood or in the bark (Pusey 1989; Urbez-Torres *et al*., 2010). Ascospores of then called *Botryosphaeria* species were mostly found on peach shoots in a study by Pusey (1989) where the increase of airborne ascospores released were directly correlated with wetness. Conidia of *B. dothidea* were spread in orchards by mainly rainwater (Pusey, 1989; Ogawa *et al.*, 1995). Conidia of Diaporthales causing constriction canker are exuded during moist conditions from pycnidia which forms in infected woody tissue (Ogawa *et al.*, 1995). The spores can spread aerially and germinate on moist surfaces (Ogawa *et al.*, 1995). Late in the winter when perithecia of *Eutypa lata* are mature, ascospores are released and dispersed by wind and rain to cause Eutypa dieback in stone fruit trees (Ogawa *et al.*, 1995). Conidia of *L. cincta* and *L. persoonii* are the primary inoculum for causing Leucostoma canker and are most abundant in late fall and early spring when the conditions are cool and moist (Ogawa *et al.*, 1995).

Basidiomycetes fungi can form fruiting bodies in the form of basidiocarps on branches and stems of dead trees or infected living trees (Ogawa *et al.*, 1995; Takemoto *et al.*, 2010). These structures release basidiospores into the air which can then land on open wounds (Ogawa *et al.*, 1995; James and Vilgalys, 2001). The basidiospores will germinate inside the xylem vessels and spread inside the woody tissue (Ogawa *et al.*, 1995).

These spores of the canker and wood rot pathogens can then enter the hosts through pruning wounds or any other wounds (Pusey, 1989; Mehl *et al.,* 2013). It is known that Botryosphaeriaceae can enter their hosts through natural openings such as stomata and lenticels and live in the host endophytically (Michailides, 1991). Spores can also land between bud scales and in favourable conditions, infection of the entire bud can be a result (Michailides, 1991). For infection with fungi such as *Cytospora* spp. to occur, the presence of free water is required (Bertrand and English, 1976). Species of Botryosphaeriaceae can enter the cortex of a plant after infection and invade the xylem, which can completely block the vessels (Mehl *et al.*, 2013). These pathogens can remain latent inside the healthy tissue without showing any symptoms on the host until the plant experience stress conditions due to factors other than the pathogen infection itself (Smit *et al.*, 1996).

In a recent study done by Moyo *et al.* (2014), *Aplosporella prunicola* was recovered from arthropods on grapevines. This suggests that fungal species associated with dieback of stone fruit trees were also present on grapevines and found on arthropods which can spread

the disease by carrying spores to other pruning wounds and openings (Damm *et al.,* 2007b; Moyo *et al.*, 2014).

After colonization of the wood by the pathogens, the twigs, branches and stems will then start showing symptoms of dieback and cankers will start to appear in the infected areas (Slippers and Wingfield, 2007). If the infected areas are not removed from the trees as well as from the orchards, the pathogen will develop overwintering structures and in favourable conditions, the spores will be released again and more infection will take place (Bertrand and English, 1976; Van Zyl, 2011). Therefore the removal of wood after pruning and cutting off the parts of a tree which shows dieback are very important to keep inoculum levels as low as possible in orchards.

MANAGEMENT STRATEGIES TO CONTROL CANKER AND WOOD ROT
Cultural practices

When new orchards are established, certified plant material with blue labels should be used to be sure that the plant material are visually free of any diseases (Mostert *et al.*, 2016b). Even though trees are visually free from diseases, latent infections can occur which cause pathogens to be spread widely throughout the growing regions (Mehl *et al.*, 2013).

The use of tissue culture techniques, a method known for making new plants to use for propagation, have recently been applied at a great extent for a number of reasons which include, among other things, the elimination of viruses and diseases from the trees being propagated (Paunovic *et al*., 2007). By growing plants *in vitro,* it is referred to as micropropagation (Carrasco *et al.*, 2013). Micropropagation are used to produce plants that are identical according to genetics, normal and uniform in development and physiology and free of any pathogens, while the time needed to produce the plants are less (Rathore *et al*., 2004). The possibility of using micropropagation for stone fruit crops to produce disease free trees was reviewed by Carrasco *et al.* (2013). Rootstock cuttings that are harvested from infected mother blocks can spread diseases into new orchards (Vujović *et al.,* 2012). By using and producing tissue culture rootstock plants, both the industry and nurseries will be ensured of good quality, uniform and pathogen free plants (García-Gonzáles *et al.,* 2010).

Stone fruit orchards are being pruned every year to keep the tree in the correct position for optimal production and also removing the dead or excess wood (Bertrand and English, 1976). After pruning, all the shoots and branches should be removed from the orchard and burned (Van Zyl, 2011) as it can be a source of inoculum where Ascomycetes and Basidiomycetes can form their fruiting bodies (Bertrand and English, 1976). Sanitation practices should be applied to remove or reduce inoculum sources from orchards to slow down the epidemic and also to decrease the frequency and the severity of the disease (Carter, 1983).

Practices such as the time of pruning can influence the risk of getting infections in an orchard (Carter, 1983). Pruning should not be done in periods when frequent rainfall occur as spores are easily released and transmitted by water (Bertrand and English, 1976; Van Zyl, 2011). When branches and twigs are being pruned off, the angle of the cut should be 45º to make sure that excess water can run off easily (Matthee and Thomas, 1977b). If water does not run off and it accumulates on the surface, spores can also gather on the open wound in the water and infect the wound (Matthee and Thomas, 1977b). It is very important to make sure that the tools and implements that are used, are sanitized as pathogens can be distributed with these implements and tools (Van Zyl, 2011).

Grapevine and pome fruit trees can be alternative hosts for stone fruit pathogens (Mostert *et al.,* 2016a). More often, stone fruit orchards are in close proximity of vineyards. Care should be taken that vineyard sanitation practices are also well applied, to prevent inoculum build up on dead plant material.

Wound protection

Since pruning wounds are important sites for infection, it is important to protect these wounds. For a pruning wound protectant to work optimally, the three factors that contribute to the success thereof are to select the most effective pruning wound protectant, to apply it correctly and to apply it at the most efficient time (Matthee and Thomas, 1977b).

Ease of application and effective coverage are important factors to take in consideration when choosing a pruning wound product (Matthee and Thomas, 1977b). To inhibit the pathogens causing dieback to enter the pruning wounds, the wound protectants should contain a suitable fungicide (Matthee and Thomas, 1977b). The protectant should be able to resist any unfavourable weather circumstances such as rain or sun that may cause the protectant to wash off or crack (Matthee and Thomas, 1977b). A wound protectant which is not phytotoxic should be used and which also can speed up the bark wound response to promote callus formation and thereby reduce the chances of getting infections (Matthee and Thomas, 1977b; Biggs, 1990). The correct application of the product is as important as choosing the correct product, since incorrect application of a good protectant will not give adequate protection or control (Matthee and Thomas, 1977b).

Fungicides can be used to protect wounds made during the pruning of stone fruit trees against the infection of different wound invading pathogens (Spiers and Brewster, 1997). However, there are currently no curative fungicides available to heal infections made by canker pathogens (Van Zyl, 2011). A recent study done by Olmo *et al*. (2017) suggested that thiophanate-methyl fungicide can successfully be used to control pathogens from the Botryosphaeriaceae on almond trees.

Another option for pruning wound protection could be biological control. A study done on grapevines showed that the use of biological control agents can offer an extended period of protection in comparison to applying fungicides to grapevine pruning wounds (Kotze *et al*., 2011). When *Trichoderma* species are used as biological control, they have the ability to colonize grapevine pruning wounds and survive during unfavourable conditions and therefore protecting the wounds against pathogens for a longer term (Kotze *et al.*, 2011). There are several modes of action which enable *Trichoderma* species to persist in wood, of which the most important one to colonize the wood, is competitive exclusion (Mutawila *et al*., 2011). The possibility of using *Trichoderma* spp. or other biological control agents against canker and wood rot pathogens on stone fruit trees, should be studied further.

PROPAGATION PROCESSES

Understanding the propagation process of stone fruit trees will aid in identifying areas where canker and wood rot pathogens can enter young trees. A recent study on apple nursery trees showed that 38% of nursery trees isolated from had canker pathogens present in the bud union (Havenga, 2017). The production of disease free nursery trees are very important and therefore starting with clean disease free propagation material is important. There are different propagation methods that can be applied to produce a new tree. Either budding or grafting can be used for stone fruit trees, although budding is the most common method used (Kumar, 2011). For any type of budding or grafting there are rootstock and scion material needed. This propagation material needs to be free of any diseases and viruses to ensure that the propagation process will be successful when the trees are sold and planted (Theron and Steyn, 2016). Nurseries get the scion and rootstock material from mother block orchards which are managed by Plant Improvement Organizations (PIO`s) (Mostert *et al.*, 2016b). If employees of the PIO`s meet the requirements of the scheme`s standard operating procedure, they can act as internal inspectors to make sure that all the blocks that are registered under their PIO comply with the phytosanitary requirements of the Deciduous Fruit Plant Improvement Association (DPA) scheme (PlantSA, 2017).

Budding

The two most used budding methods by nurseries in the Western Cape are T-budding and chip budding. There are also two different times when propagation can be done, either in the dormant season in the winter or in the growing season in spring. In the dormant season, the chip budding method is used and in the growing season, either T-budding or chip budding is used (Crasweller, 2005). In the budding process, only one scion bud is used rather than a piece of the scion shoot with a number of buds as in the case of grafting (Crasweller, 2005).

T-budding

During the period when T-budding is done, the appearance of slipping bark can be seen when the bark can easily be lifted or removed from the shoot (Crasweller, 2005). The rootstock can be either a 1-year-old seedling or a rooted rootstock. The propagation process starts where a "T" is cut into the bark of the rootstock and a scion bud, which is cut from the scion shoot, is placed in the T-cut (Crasweller, 2005). The bud is then wrapped with budding tape to stay in place and the rootstock is cut just above the inserted bud only in the next growing season in spring (Crasweller, 2005).

Chip budding

Chip budding is used when the bark is not slippery and when the scion and rootstock are both still totally dormant (Crasweller, 2005). According to Crasweller (2005) the chip budding method is used more frequently as the bud tend to grow out better. On the scion shoot, the bud is cut out by making a cut below the bud down into the wood at a slight angle and making a second cut from above the bud going down to the first cut, taking the bud together with scion wood as well (Crasweller, 2005). The same cuts are made in the rootstock and the bud is inserted and wrapped securely with budding tape to make sure that it does not dry out (Crasweller, 2005). The rootstock is cut back when the bud union is complete (Crasweller, 2005).

Factors affecting the success of the budding process

There are a few factors that may affect the success of the budding process in order to produce a good quality nursery tree. First of all, the success of budding depends on the skills and accuracy that are applied during the process and then the influences of the plant and immediate environment (Kumar, 2011). The time of the year to do budding differs for different fruit types. The slippery bark appearance can be seen in spring and the shoots are dormant in autumn to winter seasons. The best time of the year for stone fruit propagation would be in spring or autumn to winter (Kumar, 2011). It is important to make sure that the rootstock and the scion cultivars that will be used, are compatible with each other to get high success in the growth and uniting of the two plant parts (Kumar, 2011). The age of the plant material also plays a role in the success of the process. The rootstock should be younger than two years and the scion material one to two years old to ensure successful budding (Kumar, 2011). The orientation of the scion bud onto the rootstock should be orientated as it grows normally to form a successful bud union (Kumar, 2011). After the bud is inserted on the rootstock, care should be taken to make sure that the union does not dry out by wrapping it securely with a protective material (Kumar, 2011). Enough soil moisture after the budding process is important for good cambial activity to ensure success of the bud union (Kumar, 2011).

Nursery trees

After the nurseries have done the budding process, the new plants grow for one season at the nurseries after which it is being lifted in July to August as a dormant nursery tree. It is then bundled into different size categories according to the diameter of the trunk just above the bud union (Theron and Steyn, 2016). The size classification used in South Africa for the tree categories include Tall Large (15 mm+ and more than 1.8 m), Large (15 mm+ and less than 1.8 m), First size (12 – 15 mm), Medium (10 – 12 mm), Standard (8 – 10 mm) and Small (7 mm) (Theron and Steyn, 2016). The DPA will inspect trees for sufficient above and below soil growth as well as strong attachment at the bud union and that no external disease symptoms are present (Van Rensburg, 1997).

Trees get labelled with a blue certification label which ensures that the plant material are checked and meet the minimum requirements of the DPA scheme (Van Rensburg, 1997; Mostert *et al.*, 2016b). It is very important for nurseries to sell high quality nursery trees to the industry as plant material can make up to 30% of the establishment costs of new orchards. When nursery trees are being established in commercial orchards, the physical health and quality of the trees need to be of high standard for the trees to be successfully established. The shoot to root ratio together with the root quality, quantity and morphology as well as the tree size, bud quality and the physical appearance and injuries of the tree are the main characteristics that determine if a nursery tree is of good quality (Theron and Steyn, 2016).

CONCLUSION

Canker and wood rot pathogens can easily infect a tree through wounds and natural openings, colonize the parts of the tree and cause dieback of the different plant parts or cause death of the tree, especially in younger trees (Matthee and Thomas, 1977a; Slippers and Wingfield, 2007). A recent study on apple trees identified the same pathogens on propagation material, certified nursery trees as well as diseased 1-year-old trees from commercial orchards (Havenga, 2017). The nursery trees that are sold to farmers does not show any symptoms of disease, however, as soon as the trees are being established and experience stressfull conditions, the disease develop and symptoms including dieback, cankers and wood rot become visible (Havenga, 2017).

Decline and death of young stone fruit trees have regularly been seen in the industry. The phytosanitary status in regards to canker and wood rot pathogens on stone fruit nursery trees and propagation material is unknown. Knowledge regarding latent infections in visually clean material will enable further research into the application of management strategies to ensure cleaner nursery trees sold to producers.

13

AIM AND OBJECTIVES

The aim of the study was to identify the fungal canker and wood rot pathogens present in propagation material and nursery stone fruit trees as well as evaluating the pathogenicity of possible canker and wood rot pathogens. The objectives were:

1. To identify the fungal canker and wood rot pathogens present in scion and rootstock stone fruit propagation material;

2. To identify the fungal canker and wood rot pathogens present in nursery stone fruit trees and

3. To evaluate the pathogenicity of possible canker and wood rot pathogens.

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TABLES AND FIGURES

Table 1. Distribution percentage per stone fruit crop type.

Crop	Crop Distribution (%) [a]			
	Exports	**Local Market**	**Processed**	**Dried**
Apricots	8	4	73	14
Peaches and Nectarines	6	21	67	6
Plums	74	23	3	-
Cherries	35	56	9	-

[a] Data obtained from Hortgro (2017).

Table 2. Largest export markets per stone fruit crop type.

Crop	Export Markets (%) [a]			
	Middle East	**United Kingdom**	**Europe**	**Far East and Asia**
Apricots	46	27	25	1
Peaches	42	36	15	2
Nectarines	17	57	22	1
Plums	18	26	45	5
Cherries	19	50	7	17

[a] Data obtained from Hortgro (2017).

Table 3. Incidence of fungal pathogens isolated from 170 stone fruit trees by the Disease Clinic (Plant Pathology, Stellenbosch University) from 2007 to 2018.

Pathogens [a]	Number of pathogens isolated from trees
Botryosphaeriaceae	62
Leucostoma spp.	45
Diaporthe spp.	30
Phaeoacremonium spp.	27
Tympanidaceae	26
Basidiomycetes	25
Colletotrichum spp.	10
Eutypa spp.	9
Cytospora spp.	7
Didymosphaeria spp.	2
Calospaeria spp.	2
Phoma spp.	2
Phaeomoniella spp.	1
Ceriporia spp.	1
Cryptosporiopsis spp.	1
Phialophora spp.	1

[a] Fungal isolates were identified to genus level. The genera identified in the Botryosphaeriaceae and in the Basidiomycetes have been grouped together.

Table 4. Different stone fruit tree types infected with fungal pathogens isolated by the Disease Clinic (Plant Pathology, Stellenbosch University) from 2007 to 2018.

Stone fruit trees	Number of pathogens isolated
Plum	64
Peach	50
Nectarine	28
Cherry	15
Apricot	13

CHAPTER 2

Occurrence of canker and wood rot pathogens on stone fruit propagation material and stone fruit nursery trees in South Africa

ABSTRACT

Young stone fruit trees with symptoms of dieback or canker, could result in the death of young trees. One source of inoculum could be through nursery trees harboring latent infections. The phytosanitary status of stone fruit nursery trees in South Africa is not known, since canker and wood rot pathogens could be present inside the trees and not visible to inspectors. The objectives of this study were to identify the fungal pathogens present in nursery stone fruit trees as well as propagation material and to evaluate their pathogenicity. Isolations were made from scion and rootstock propagation material and from certified nursery stone fruit trees. Different plum and nectarine cultivars investigated included three plum scion cultivars on three plum rootstock cultivars and one nectarine scion cultivar on two nectarine rootstock cultivars. The plant material sampled did not have any external symptoms. The certified nursery trees when cross-sectioned displayed brown discoloration from the pruning wound, bud union and often from the crown. Fungal species isolated were identified by sequencing of the relevant barcoding genes and phylogenetic analyses thereof. Canker and wood rot associated fungi as well as "*Cylindrocarpon*"-like fungi were identified. Buds used for occulation had low levels of infection, with 1.2% of dormant buds infected and 0.4% of green buds infected. The dormant rootstock shoots had infection with canker pathogens (6.2%) before it was planted in the nursery fields and increased as the ungrafted, rooted rootstock plants had 10.6% infection with canker and wood rot pathogens and 6.4% infection with "*Cylindrocarpon*"-like fungi. Out of 1080 nursery trees, the canker and wood rot associated fungi infected 21.8% of trees and the "*Cylindrocarpon*"-like fungi infected 23.6% of trees. The canker causing pathogens that were isolated the most were *Cadophora luteo-olivacea* and *Diplodia seriata*. Of the "*Cylindrocarpon*"-like fungi *Dactylonectria novozelandica* and *Dactylonectria torresensis* occurred the most. A low incidence of wood rot fungi was found with only 1.5% of nursery trees infected. In total 26 new reports of fungal species on stone fruit in South Africa were made. Of these, 22 have also not been found on stone fruit world-wide. Ten new species were found that would need to be described. The pathogenicity trials' results confirmed the pathogenic status of the canker and wood rot causing species. *Lasiodiplodia theobromae* was the most virulent species in both plum cultivars in the two orchard trials. The pathogen status of the "*Cylindrocarpon*"-like fungi remains to be determined. The results of this research show that nursery stone fruit trees and propagation material harbor latent infections. Different

management practices need to be evaluated to prevent these infections and ensure disease free stone fruit nursery trees.

INTRODUCTION

Canker and wood rot pathogens can infect fruit trees and cause symptoms such as dieback, cankers, blight, gummosis and wood rotting (Ogawa *et al*., 1995; Matthee and Thomas, 1977a; Damm *et al*., 2007a; Cloete *et al*., 2011; Gramaje *et al*., 2012). Several studies done in South Africa have identified fungi associated with dieback, cankers and wood necroses of stone fruit trees. Species of *Aplosporella*, *Calosphaeria*, *Collophorina*, *Coniochaeta*, *Cryptovalsa*, *Diplodia*, *Diaporthe*, *Didymosphaeria*, *Dothiorella*, *Eutypa*, *Jattaea, Lasiodiplodia, Leucostoma, Neofusicoccum, Paraconiothyrium, Phaeoacremonium* and *Phaeomoniella* have been associated with necrotic wood tissue and dieback symptoms of stone fruit trees in South Africa (Smit *et al*., 1996; Crous *et al*., 2000; Damm *et al*., 2007a,b; Damm *et al*., 2008a,b,c; Damm *et al*., 2010; Jami *et al*., 2017; Moyo *et al*., 2018; Spies *et al*., 2018). The more recent investigations focusing on dieback and cankers of stone fruit trees identified associated pathogens from several areas in the Western Cape (Bonnievale, Franschhoek, Montagu, Paarl, Robertson, Stellenbosch, Tulbagh) as well as Limpopo (Modimolle, Mookgopong) (Damm *et al*., 2007a,b; Damm *et al*., 2008a,b,c; Damm *et al*., 2010). The most isolates (67) were reported from species of the Botryosphaeriaceae with *Diplodia seriata* De Not. being the dominant species isolated from apricot, nectarine, peach and plum wood from the Western Cape and Limpopo regions (Damm *et al*., 2007a). Wood rot of stone fruit trees in South Africa have been associated with species of the genera *Armillaria*, *Chondrostereum*, *Schizophyllum* and *Trametes* (Doidge, 1950; Ogawa *et al*., 1995; Crous *et al*., 2000).

Canker and wood rot pathogens can enter and infect the host through wounds, which results in colonization of vascular tissues and subsequently blockage thereof (Mehl *et al*., 2013). This then leads to dieback of parts of the tree such as the shoots, branches and even the main trunk, which can lead to the death of the tree in severe cases (Slippers and Wingfield, 2007). Some fungal pathogens can also live as endophytes in the hosts where they could have entered through wounds or natural openings (Slippers and Wingfield, 2007). Infection of young trees result in poor growth of the trees together with low yields. When dieback and death of young trees are observed in newly planted orchards, re-establishing of new trees need to be done, which leads to increased establishment costs (Smit *et al*., 1996).

Nursery apple trees can harbour latent infections of canker and wood rot pathogens (Havenga, 2017). Symptoms due to latent infections in young trees are only seen when the trees are planted out in commercial orchards coupled with the exposure to stress conditions (Smit *et al.*, 1996; Marek *et al*., 2013). Typical stress conditions are practices which include the propagation process, uplifting the trees and cold storage, the establishment in a new

orchard, water and nutrient availability and further incorrect cultural practices (Steyn *et al*., 2016). During the nursery process the trees experience less stress in comparison to when they are lifted at the nurseries, sometimes kept in cold storage and then planted out in suboptimal conditions in the new orchard.

Over the past 11 years, the Disease Clinic of Stellenbosch University, analysed 170 young stone fruit trees from which canker and/or wood rot pathogens were isolated. These trees represent 170 commercial stone fruit orchards in the Western Cape having symptoms of cankers or wood rot. It is known, that fungal infections can be one of the causal reasons for death of young stone fruit trees observed in South Africa (pers. comm. Piet Stassen stone fruit consultant). Additionally, symptoms of brown discolouration at the base of the trees have been frequently observed in the stone fruit industry.

The presence of latent infections of canker and wood rot pathogens within certified nursery stone fruit trees in South Africa is not known. Therefore, the aim of this study was to evaluate the phytosanitary status of nursery stone fruit trees as well as the propagation material, to understand where infection of canker and wood rot pathogens could occur. The occurrence of canker and wood rot pathogens were assessed in 1) scion stone fruit propagation material; 2) rootstock stone fruit propagation material, and 3) nursery stone fruit trees. Furthermore, the pathogenicity of the newly reported canker and wood rot species on stone fruit were evaluated in field trials.

MATERIALS AND METHODS

Sampling of stone fruit plant material

Scion material
Scion shoots were sampled from stone fruit mother trees in the Western Cape. Green shoots were cut in January 2017 and dormant shoots in May 2017 from the same blocks. For a cultivar, 120 shoots were sampled from two blocks, 60 shoots per block. The sampling was done in collaboration with the South African Plant Improvement Organization (SAPO). Three plum scion cultivars and one nectarine scion cultivar were sampled for the green scion material and only three plum cultivars were sampled for the dormant scion material. Only green buds were used for nectarine trees. Isolations were made from the green scion shoots after sampling. The dormant scion shoots were cut in May and kept in cold storage (4ºC) until October 2017, as is the standard procedure in industry.

Rootstock material
The rootstock plant material consisted of the ungrafted, rooted rootstock plants that were collected from three nurseries and the dormant rootstock shoots cut from mother trees from

SAPO. The ungrafted, rooted rootstock plants were sampled in January 2017. A total of 120 plants per cultivar were sampled. Forty plants (where available) were sampled for three plum and two nectarine cultivars. The dormant rootstock shoots were cut from the mother trees in May 2017. A total of 180 shoots were sampled from three mother blocks, 60 shoots per site. Three plum and one nectarine rootstock cultivar were sampled. The material were stored at 4ºC until completion of isolations.

Nursery trees

Nursery plants with different scion and rootstock combinations were collected in August 2017 from three nurseries in the Western Cape. The scion and rootstock cultivars were the same as used for the scion and rootstock material. A total of 120 trees per scion and rootstock combination were collected (Table 1). The only exception is the combination Plum 1-3,2-3,3-3 which was from one nursery where 40 plants were sampled of the three scion cultivars on Plum 3 rootstock. This rootstock was only found at one nursery and the three combinations were therefore pooled together. Additionally, 20 trees made from tissue culture rootstocks and 20 trees made from hardwood rootstock cuttings were also sampled. This was done to compare the phytosanitary quality of tissue culture rootstocks versus conventional hardwood cutting. Only a limited number of tissue culture plants were available, because of high demand for these trees in the industry.

Isolations from plant material

Scion material

The scion shoots were prepared by removing all the leaves from the shoots and triple surface sterilized by soaking the shoots in 70% ethanol solution for 30 seconds, then in 1% NaOCl solution for 60 seconds and lastly in 70% ethanol solution for 30 seconds. The shoots were left to air dry on sterile tissue paper in a laminar flow cabinet. From the green shoots as well as from the dormant shoots eight buds were cut off in the same way that the nurseries would cut buds for occulation. The buds were placed onto 2% Potato Dextrose Agar (Biolab, Midrand) amended with streptomycin sulphate (40 mg/L, Calbiochem, Merck) (PDA+s). Four buds were placed onto one PDA+s Petri dish, thus two Petri dishes per shoot. The dishes were incubated at 23ºC under natural light for two weeks or until sufficient fungal growth were seen. Subcultures were made from representative primary isolations and incubated at the same conditions.

Rootstock material

The ungrafted, rooted rootstock plants were prepared by removing the excess roots and washing the soil off. The rootstock plants were also triple surface sterilized as described for

the scion shoots. Isolations were made from the rootstock plants by splitting the tip and crown and scraping the bark off wounds on the rootstock. Four wood pieces were isolated from the tip and four wood pieces were isolated from wounds, if present on the rootstock plant, and put onto two PDA+s Petri dishes. From the crown, eight pieces were isolated 2 cm from the edge of the crown and put onto two PDA+s Petri dishes.

For the dormant rootstock shoots, preparations were done as described for the scion shoots and isolations were made from the nodes and internodes of each shoot. Four disks from the nodes and four disks from the internodes were cut using a flame sterilized pruning shear. The four node disks were put onto one PDA+s dish and the four internode disks were put onto another PDA+s dish. All the dishes were incubated under the same conditions as for the scion material and subcultures were made from representative primary isolations and incubated at the same conditions.

Nursery trees

The nursery trees were prepared for isolations by cutting off the roots and some of the upper growth above the bud union. Isolations were made from three plant parts on the tree, namely the crown, bud union and rootstock pruning wound. The three plant parts were split and triple surface sterilized as described for the scion material, but 3% NaOCl was used instead. From each of the three plant parts, eight pieces from the margin of the discoloured vascular tissue were isolated and placed onto two PDA+s dishes. Where no symptoms were observed, isolations were made from the healthy tissue. All the dishes were incubated under the same conditions as for the scion material and subcultures were made from representative primary isolations and incubated at the same conditions.

Identification of fungal species

All the isolates were grouped into cultural growth groups according to differences observed in characteristics of the growth of the colony with regards to colour, shape, texture and size. Additionally, conidia shape, size and colour were observed where possible, under a microscope. Fungal groups such as Botryosphaeriaceae, Diaporthales, *Phaeoacremonium* and "*Cylindrocarpon*"- like fungi were identified. Studies on Botryosphaeriaceae by Van Niekerk *et al*. (2004) and Phillips *et al.* (2012), on Diaporthales by Udayanga *et al*. (2012, 2014) on *Phaeoacremonium* by Gramaje *et al*. (2015) and Damm *et al*. (2008b) and on "*Cylindrocarpon*"- like cultures by Agustí-Brisach and Armengol (2013) were used to aid in identifications. Unknown cultures were subjected to molecular identification. Where possible two isolates per group were chosen to confirm the identity with sequencing of the relevant barcoding gene and phylogenetic analyses. The selected isolates were stored in the culture

collection of the Department of Plant Pathology (STEU). Known saprophytes were noted and discarded.

Molecular identification

DNA extraction

Representative cultures were grown on PDA for approximately 2 weeks. The DNA isolation protocol that was described by Damm *et al*. (2008b) was used with some amendments. The fungal mycelium was scraped off and placed into 2 mL Eppendorf tubes with 0.5 mg glass beads and 600 µL CTAB (2% CTAB, 1 M Tris, pH 7.5, 5 M NaCl, 0.5 M EDTA, pH 8.0). The tubes were shaken for 5 minutes at 30 vibrations per second in a Retsch Mixer Mill MM301 (Retsch, Haan, Germany) and then incubated at 65ºC for 15 minutes in a water bath. After the incubation period, 400 µL chloroform:isoamylalcohol (24:1) were added to the tubes and it was then centrifuged for 15 minutes at 13 500 rpm. The supernatant of each tube was transferred to a new 2 mL Eppendorf tube which already contained 200 µL of ammonium acetate (7.5 M) and 600 µL isopropanol. The tubes were centrifuged for 15 minutes at 13 500 rpm and the supernatant was discarded without losing the pellet that formed at the bottom and 200 µL of 70% ethanol was added to each tube with the pellet. The tubes were centrifuged for 5 minutes and the supernatant was discarded and centrifuged for another 1 minute at 13 500 rpm. The last bit of supernatant that was still in the tubes were removed by using a pipet and drying the tubes for 2 – 3 minutes in a heating block. The DNA pellets were dissolved in 200 µL double distilled water (ddH$_2$O).

Polymerase chain reaction (PCR) and electrophoresis

For specific taxonomic groups, there were specific primers selected for amplification. For the Amphisphaeriaceae, Basidiomycetes, Calosphaeriaceae, Tympanidaceae, Coniochaetaceae, Diaporthaceae, Diatrypaceae, Didymellaceae, Didymosphaeriaceae, *Neophaeomoniella* sp., Phaeosphaeriaceae, Valsaceae and Xylariaceae the internal transcribe spacers (ITS) 1 and 2 and the 5.8S rDNA gene area were amplified with ITS-5F and ITS-4R primers (White *et al*., 1990). The total reaction volume was 20 µL and the PCR reaction contained 1 µL of DNA, 10 µL 2x KAPA Taq ready mix (KAPABiosystems, Massachusetts, United States), 0.8 µL ITS-5 (0.4 pmol/µL), 0.8 µL ITS-4 (0.4 pmol/µL) and 7.4 µL ddH$_2$O. The reaction conditions that were used consisted out of an initial denaturation step for 5 minutes at 94ºC, followed by 30 cycles of 94ºC for 30 seconds, 55ºC for 30 seconds and 72ºC for 30 seconds with a final extension step for 7 minutes at 72ºC.

For the Botryosphaeriaceae and Diaporthaceae, the elongation factor 1-alpha gene (EF-1 α) was amplified with primers EF-1 728F and EF-1 968R (Carbone and Kohn, 1999).

The same reaction volumes were used as previously described. The PCR conditions include an initial denaturation step for 5 minutes at 94ºC, followed by 30 cycles of 94ºC for 45 second, 53ºC for 45 seconds and 72ºC for 90 seconds with a final extension step for 7 minutes at 72ºC. *Diplodia seriata* was identified by using the species-specific primers DS3.8 S3 and DS3.8 R6 (Martín *et al*., 2014). A total reaction volume of 20 µL containing 1 µL of DNA, 8 µL 2x KAPA Taq ready mix, 0.8 µL DS3.8 S3 (0.4 pmol/µL), 0.8 µL DS3.8 R6 (0.4 pmol/µl) and 9.4 µL ddH$_2$O. The PCR conditions were an initial denaturation step for 5 minutes at 95ºC, followed by 30 cycles of 94ºC for 30 seconds, 57ºC for 45 seconds and 72ºC for 45 seconds with a final extension step for 7 minutes at 72ºC. Representatives were sequenced to confirm the species identity by using the EF-1 728F and EF-1 968R primers.

Species of *Cadophora* were identified with amplifying the ITS regions with ITS-5F and ITS-4R primers as described above. Primers designed by Alves *et al*. (2008) (EF1-668F and EF1-1251R) were also used to amplify part of the EF-1 α region. The reaction volumes and conditions for the EF primers were the same as described for the Botryosphaeriaceae species.

The *Phaeoacremonium* species were identified using partial β-tubulin gene amplified by the primers T1 (O'Donnell and Cigelnik, 1997) and Bt2B (Glass and Donaldson, 1995). The same reaction volumes were used as previously described. The PCR reaction conditions consisted out of an initial denaturation step for 5 minutes at 94ºC followed by 36 cycles at 94ºC for 45 seconds, 55ºC for 45 seconds and 72ºC for 90 seconds and a final extension step for 6 minutes at 72ºC. Screening were done for *Phaeoacremonium parasiticum* using species-specific primers T1 and Pbr2_2 (Mostert *et al*., 2006) and for *Phaeoacremonium minimum* using primers T1 and Pbr6_1 (Mostert *et al*., 2006) with PCR reaction volumes as described previously and step-down PCR conditions as follow: 5 minutes at 94ºC with 5 cycles of 94ºC for 30 seconds, 66ºC for 30 seconds and 72ºC for 60 seconds; 5 cycles of 94ºC for 30 seconds, 64ºC for 30 seconds and 72ºC for 60 seconds and lastly 25 cycles of 94ºC for 30 seconds, 62ºC for 30 seconds and 72ºC for 60 seconds with a final extension step of 72ºC for 6 minutes. Representatives were sequenced to confirm the species identity by using the T1 and Bt2B primers.

The "*Cylindrocarpon*"-like fungi were identified with sequencing of the histone gene. The primers CYLH3F and CYLH3R were used to amplify the partial histone H3 gene according to Crous *et al*. (2004). The PCR reaction volumes were as described previously for the ITS gene and the PCR conditions consisted out of an initial denaturation step for 5 minutes at 96ºC followed by 30 cycles at 96ºC for 30 seconds, 52ºC for 30 seconds and 72ºC for 60 seconds and a final extension step for 5 minutes at 72ºC.

All the PCR reactions were done by using an Applied Biosystems 2700 PCR machine (Carlsbad, California, USA). The PCR products were separated using electrophoresis 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 ethidium bromide. The gel was visualized under ultraviolet (UV) light with the GeneGenius Gel Documentation and Analysis System (Syngene, UK) together with a 100-bp DNA ladder (GeneRuler, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Sequencing of PCR products

Before forward and reverse sequencing could be done, the PCR products were purified with the use of the MSB Spin PCRapase kit (Invitek, Berlin, Germany). The Thermocycler conditions for the sequence reaction were 95ºC for 1 minute followed by 30 cycles of 95ºC for 10 seconds, 50ºC for 5 seconds and 60ºC for 4 minutes and a final extension of 60ºC for 30 seconds. The gene areas were sequenced using ABI PRISM Big Dye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, California, United States) with the primers that were used in the initial PCR reactions. The nucleotide order of samples were read in an ABI 3130xl DNA sequencer (Perkin-Elmer, Norwalk, California, United States) at the DNA Sequencing Unit at the Central Analytical Facility (CAF) of Stellenbosch University.

Phylogeny

By using Geneious R10.1.3. (Biomatters Ltd., Auckland, New Zealand) the forward and reverse sequences for each isolate were edited, aligned and consensus sequences extracted. The identities of the consensus sequences were determined through the Basic Local Alignment Search Tool (BLAST) of the National Centre for Biotechnology Information's (NCBI) nucleotide database (http://www.ncbi.nlm.nih.gov/Genbank). Reference sequences for each taxonomic group were obtained from GenBank and aligned with representative sequences from this study using the MAFFT V7.222 program with the L-INS-I method (Katoh *et al*., 2002) in Geneious R10.1.3. Maximum likelihood (ML) analysis were done using PHyML (Guindon and Gascuel, 2003) under the general time reversible (GTR) model. The gamma distribution and proportion of invariable sites were assessed. A 100 replicates were used to calculate the bootstrap support values and the clades with a bootstrap value of equal or more than 70% were considered to be significant and highly supported (Hillis and Bull, 1993). The ex-type isolates included in the phylogenetic trees are indicated with a "T", where available.

Pathogenicity test

Canker and wood rot pathogens

A total of 66 isolates, representing 38 species (where possible, two isolates per species) of the total of 55 canker and wood rot species identified, were included in the pathogenicity trial. Seventeen of the 55 species were not tested in the pathogenicity trial, due to low incidence or have been tested in pathogenicity trials on stone fruit by Damm *et al.* (2007a, 2008b, 2010). These include *Cadophora spadicis* Travadon, D.P. Lawr., Roon.-Lath., Gubler, W.F. Wilcox,

Rolsh. & K. Baumgartner, *Cadophora* sp. 1, *Coniochaeta prunicola* Damm & Crous, *Coniochaeta* sp. 3, *Coprinellus flocculosus* (DC.) Vilgalys, Hopple & Jacq. Johnson, *Coprinellus micaceus* (Bull.) Vilgalys, Hopple & Jacq. Johnson, *Didymella americana* (Morgan-Jones & J.F. White) Qian Chen & L. Cai, *Neofusicoccum australe* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, *Neopestalotiopsis javaensis* Maharachch., K.D. Hyde & Crous , *Neophaeomoniella zymoides* (Hyang B. Lee, J.Y. Park, Summerb. & H.S. Jung) Crous, *Paraphoma radicina* (McAlpine) Morgan-Jones & J.F. White, *Peniophora* sp., *Phaeoacremonium australiense* L. Mostert, Summerb. & Crous, *Phaeoacremonium fraxinopennsylvanicum* (T.E. Hinds) Gramaje, L. Mostert & Crous, *Phaeoacremonium minimum* (Tul. & C. Tul.) Gramaje, L. Mostert & Crous, *Phaeoacremonium scolyti* L. Mostert, Summerb. & Crous and *Truncatella restionacearum* S.J. Lee & Crous. The trial layout was designed as an incomplete block design. Two plum orchards were used, African Rose (Orchard 1) and Sunkiss (Orchard 2). Both orchards were planted in 2008 and are situated in Simondium, Western Cape. These cultivars were chosen for sufficient length and thickness of the two to three year old shoots on the trees. Both cultivars are also of economic importance to the industry (Hortgro, 2017). Two isolates per species, where available, were plated out onto PDA and incubated for 2 weeks. Five isolates of *Diplodia seriata* were included, because three of the isolates were initially identified as *Diplodia* species. Non-colonised PDA was used as negative control. Inoculations were made on two- to three-year-old wood. Each isolate was inoculated onto 10 shoots in each orchard. The shoots were surface sterilized by spraying 70% ethanol where the inoculation point would be. The bark from the shoot was removed with a 3 mm cork-borer, a wound was made with a 3 mm drill and a colonised 3 mm diameter agar plug was placed in the wound and covered with parafilm. After 4 months the inoculated shoots were removed from the trees. The shoots were triple surface sterilized as described above, but 3% NaOCl was used instead and split longitudinally through the wound. Lesions were measured in both directions from the wound. Isolations were done by cutting 8 pieces of wood (1 x 1 mm) from the margin of the lesion and placed onto two PDA+s Petri dishes. The dishes were incubated at 24ºC for 3 weeks and fungal growth was transferred to new PDA+s dishes and incubated the same as above. Representative isolates of each species were chosen to confirm that it is the same species inoculated, by sequencing the relevant genes for each genus and comparing with the original sequence.

Statistical analysis

For the scion and rootstock propagation material, Chi-square tests were used to test for differences in the frequencies of the parts infected (buds, plants, shoots) using Frequency Procedure (PROC FREQ) of the SAS statistical software version 9.4 (SAS Institute Inc., Cary, NC, USA). Chi-square tests were used because there were no replications in the data for the

factors. The percentage infection was calculated for each factor (cultivar). The null hypothesis was stated as "the presence of infection is independent of cultivar" and was rejected at a 5% level ($P < 0.05$).

The incidence of fungal organisms present in the different plant parts of the nursery trees and in the nursery trees per cultivar combination were expressed as a percentage of the total number of plant parts and trees infected per cultivar combination. The incidence data was subjected to analysis of variance (ANOVA) and the means were compared by using Fisher's least significant difference (LSD) value at a 5% level and a 10% significance level was used where applicable to identify trends (Ott and Longnecker, 2001). Analysis was done using SAS statistical software version 9.4.

For the analysis of the tissue culture rootstock trees and hardwood rootstock trees, there were no replications in the data and therefore Chi-square tests were used to test for differences in the frequencies of the plant parts infected with "*Cylindrocarpon*"-like fungi using Frequency Procedure (PROC FREQ) of the SAS statistical software version 9.4. The percentage infection was calculated for each combination at each plant part. The null hypothesis was stated as "the presence of infection is independent of combination and plant part" and was rejected at a 10% level ($P < 0.1$).

The pathogenicity trial layout was an incomplete block design for both orchards. The mean lesion length data for the two orchards was log transformed for improvement of the normality. The data (lesion length) was subjected to analysis of variance, each orchard separately using General Linear Models (GLM) procedure of SAS statistical software version 9.4. Student's t-least significant difference was calculated at a 5% level to compare means.

RESULTS

Representative isolates, where possible two isolates per cultural growth and morphological group (Table 2), were identified (in total 113 isolates).

Phylogenetic analyses

For each taxonomic group identified, separate phylogenetic analysis was done. In the group Amphisphaeriaceae, one isolate grouped together with *Neopestalotiopsis javaensis* (Fig. 1) and two *Truncatella* species (Fig. 2) were identified namely *Truncatella angustata* (Pers.) S. Hughes (bootstrap support of 100%) and *Truncatella restionacearum*. For both *N. javaensis* and *T. restionacearum*, the phylogenies did not form monophyletic clades for these species, however, comparing the ITS sequences 99.8% (over 547 nucleotides) and 100% (over 477 nucleotides) similarity was obtained, respectively, for these species with the reference isolates NR_145241.1 and DQ278915.

Four Basidiomycetes species were identified (Figs. 3-5) which belonged to three genera. Two *Coprinellus* species (Fig. 3) which include *Coprinellus flocculosus* (bootstrap support 89%) and *Coprinellus micaceus* (bootstrap support of 99%) were identified. The one *Peniophora* isolate clustered with *Peniophora pini* (Schleich. ex DC.) Boidin, however, with low bootstrap support and a lower sequence similarity of 93% (over a length of 604 nucleotides with MH857814 reference isolate) indicates that this isolate represent a putative new *Peniophora* sp. (Fig. 4). Only one isolate of *Schizophyllum commune* Fr. was identified in this study (bootstrap support of 79%) (Fig. 5).

Biscogniauxia mediterranea (De Not.) Kuntze (bootstrap support of 100%) and an unknown *Biscogniauxia* sp. (bootstrap support of 100%) were identified (Fig. 6). The unknown *Biscogniauxia* sp. grouped together with two other unknown *Biscogniauxia* isolates from East African mahogany (HM752510) and Chilean myrtle (JQ327868) woody hosts.

The Botryosphaeriaceae isolates resulted in the identification of species of *Diplodia*, *Lasiodiplodia*, *Dothiorella* and *Neofusicoccum* (Figs. 7 and 8). *Diplodia seriata* (bootstrap support of 76%) (Fig. 7) were the most frequently observed species in the Botryosphaeriaceae (Table 3). *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (bootstrap support of 100%) and two *Dothiorella* species, namely *Dothiorella viticola* A.J.L. Phillips & J. Luque (bootstrap support of 96%) and *Dothiorella moneti* K.M. Taylor, P.A. Barber, G.E. Hardy & T.I. Burgess (bootstrap support of 99%) were found (Fig. 7). One *Neofusicoccum* species was identified as *Neofusicoccum australe* (bootstrap support of 94%) (Fig. 8).

Five *Cadophora* species and two possible new *Cadophora* species were identified (Fig. 9). The *Cadophora* species include *Cadophora gregata* (Allington & D.W. Chamb.) T.C. Harr. & McNew (bootstrap support of 71%), *Cadophora luteo-olivacea* (J.F.H. Beyma) T.C. Harr. & McNew (bootstrap support of 75%), *Cadophora malorum* (Kidd & Beaumont) W. Gams (bootstrap support of 90%), *Cadophora novi-eboraci* Travadon, D.P. Lawr., Roon.-Lath., Gubler, W.F. Wilcox, Rolsh. & K. Baumgartner (bootstrap support of 96%) and *Cadophora spadicis* (bootstrap support of 87%). Isolate STEU 8862 (*Cadophora* sp. 1) did not form a clade with bootstrap support with any of the known *Cadophora* species and the three isolates of *Cadophora* sp. 2 did also not group with any known *Cadophora* species.

Two *Collophorina* species were identified namely *Collophorina paarla* (Damm & Crous) Damm & Crous (bootstrap support of 90%) and *Collophorina rubra* (Damm & Crous) Damm & Crous (bootstrap support of 100%) (Fig. 10). Three *Coniochaeta* species and three possible new *Coniochaeta* species were identified in this study (Fig. 11). *Coniochaeta hoffmannii* (J.F.H. Beyma) Z.U. Khan, Gené & Guarro (bootstrap support of 100%), *Coniochaeta prunicola* (bootstrap support of 100%) and *Coniochaeta velutina* (Fuckel) Cooke (bootstrap support of 95%) were identified. Isolate STEU 8873 (*Coniochaeta* sp. 1) did not group with any of the known *Coniochaeta* species and isolate STEU 8877 (*Coniochaeta* sp. 3) grouped

with *Coniochaeta ligniaria* (Grev.) Cooke, but with low bootstrap support. The three isolates of *Coniochaeta* sp. 2 (bootstrap support of 91%) formed a clade which did not group with known species.

The *Cytospora* species that were identified include *Cytospora austromontana* G.C. Adams & M.J. Wingf. (bootstrap support of 98%) and *Cytospora leucostoma* (Pers.) Sacc. (bootstrap support of 76%) (Fig. 12). Three isolates of *Cytospora* sp. 1 (bootstrap support of 76%) and two isolates of *Cytospora* sp. 2 (bootstrap support of 87%) formed clades that did not group with a high enough bootstrap with known *Cytospora* species (Fig. 12). *Valsa sordida* Nitschke (sexual morph *Cytospora chrysosperma* (Pers.) Fr.) (bootstrap support of 99%) was also identified (Fig. 12). For the *Diaporthe* species, three species were identified namely *Diaporthe ambigua* Nitschke (bootstrap support of 96%), *Diaporthe aspalathi* E. Jansen, Castl. & Crous (bootstrap support of 100%) and *Diaporthe foeniculina* (Sacc.) Udayanga & Castl. (bootstrap support of 83%) (Fig. 13).

For the Diatrypaceae, *Eutypa leptoplaca* (Durieu & Mont.) Rappaz (bootstrap support of 75%) were identified and isolate STEU 8906 (*Eutypella* sp.) grouped with *Eutypella* species, but did not form a clade with bootstrap support (Fig. 14). The Dothideomycetes that were identified include two *Didymella* species and two *Didymosphaeria* species. *Didymella americana* (bootstrap support of 64%) were identified and the two *Didymella pomorum* (Thüm.) Qian Chen & L. Cai isolates grouped together with known reference isolates, although it had lower bootstrap values (Fig. 15). *Didymosphaeria rubi-ulmifolii* Ariyaw., Camporesi & K.D. Hyde (bootstrap support of 100%) and *Didymosphaeria variabile* (Riccioni, Damm, Verkley & Crous) Ariyaw. & K.D. Hyde (bootstrap support of 99%) were also identified in this study (Fig. 16). One isolate of *Neophaeomoniella zymoides* (bootstrap support of 94%) was identified (Fig. 17). Species of *Paraphaeosphaeria* that were identified include *Paraphaeosphaeria neglecta* Verkley, Riccioni & Stielow (bootstrap support of 93%) and *Paraphaeosphaeria sporulosa* (W. Gams & Domsch) Verkley, Göker & Stielow (bootstrap support of 84%) (Fig. 18). Two *Paraphoma* species were identified as *Paraphoma chrysanthemicola* (Hollós) Gruyter, Aveskamp & Verkley (bootstrap support of 92%) and *Paraphoma radicina* (bootstrap support of 97%) (Fig. 19).

Six species of *Phaeoacremonium* were identified and include *Phaeoacremonium australiense* (bootstrap support of 100%), *Phaeoacremonium fraxinopennsylvanicum* (bootstrap support of 100%), *Phaeoacremonium iranianum* L. Mostert, Gräfenhan, W. Gams & Crous (bootstrap support of 100%), *Phaeoacremonium minimum* (bootstrap support of 100%), *Phaeoacremonium parasiticum* (Ajello, Georg & C.J.K. Wang) W. Gams, Crous & M.J. Wingf. (bootstrap support of 100%) and *Phaeoacremonium scolyti* (bootstrap support of 100%) (Fig. 20). One species of *Pleurostoma* was identified as *Pleurostoma richardsiae* (Nannf.) Réblová & Jaklitsch (bootstrap support of 100%) (Fig. 21).

The "*Cylindrocarpon*"-like fungi that were identified include species of *Campylocarpon*, *Ilyonectria*, *Dactylonectria* and *Thelonectria* (Figs. 22-24). *Campylocarpon pseudofasciculare* Halleen, Schroers & Crous (bootstrap support of 100%) was the only species of *Campylocarpon* identified (Fig. 22). Two *Ilyonectria* species and one possible new *Ilyonectria* species were identified. *Ilyonectria liriodendri* (Halleen, Rego & Crous) P. Chaverri & Salgado (bootstrap support of 96%) and *Ilyonectria robusta* (A.A. Hildebr.) A. Cabral & Crous (bootstrap support of 100%) were identified and isolate STEU 8918 (*Ilyonectria* sp.) grouped with *Ilyonectria crassa* (Wollenw.) A. Cabral & Crous, but did not have high enough bootstrap support (Fig. 23). Three *Dactylonectria* species and three possible new *Dactylonectria* species were identified. The three *Dactylonectria* species were identified as *Dactylonectria macrodidyma* (Halleen, Schroers & Crous) L. Lombard & Crous (bootstrap support of 96%), *Dactylonectria novozelandica* (A. Cabral & Crous) L. Lombard & Crous (bootstrap support of 72%), which were also the most frequently observed species of the "*Cylindrocarpon*"-like fungi (Table 3) and lastly *Dactylonectria torresensis* (A. Cabral, Rego & Crous) L. Lombard & Crous (bootstrap support of 73%) (Fig. 23). The three isolates of *Dactylonectria* sp. 1 (bootstrap support of 96%) formed a clade, but did not group with any of the known *Dactylonectria* species (Fig. 23). Isolate STEU 8897 (*Dactylonectria* sp. 2) did not group with any of the known *Dactylonectria* species, while isolate STEU 8898 (*Dactylonectria* sp. 3) grouped with *Dactylonectria estremocensis* (A. Cabral, T. Nascim. & Crous) L. Lombard & Crous, but with insufficient bootstrap support (Fig. 23). The *Thelonectria* species identified in this study include *Thelonectria truncata* Salgado & P. Chaverri (bootstrap support of 99%) and *Thelonectria veuillotiana* (Roum. & Sacc.) P. Chaverri & Salgado (bootstrap support of 92%) (Fig. 24). Two possible new *Thelonectria* species which formed clades separate from any other known *Thelonectria* species were also identified namely *Thelonectria* sp. 1 (bootstrap support 100%) and *Thelonectria* sp. 2 (bootstrap support of 91%) (Fig. 24).

Diversity of fungal taxa

The 69 fungal species include canker pathogens (51 Ascomycetes), wood rot fungi (4 Basidiomycetes) and "*Cylindrocarpon*"-like fungi (14 Ascomycetes). From the scion and rootstock material and nursery trees, the species identified in this study, belonged to species within the genera *Biscogniauxia*, *Cadophora*, *Collophorina*, *Coniochaeta*, *Coprinellus*, *Cytospora*, *Diaporthe*, *Didymella*, *Didymosphaeria*, *Diplodia*, *Dothiorella*, *Eutypa*, *Eutypella*, *Lasiodiplodia*, *Neofusicoccum*, *Neopestalotiopsis*, *Neophaeomoniella*, *Paraphaeosphaeria*, *Paraphoma*, *Peniophora*, *Phaeoacremonium*, *Pleurostoma*, *Schizophyllum*, *Truncatella*, *Valsa* and species from the "*Cylindrocarpon*"-like fungi (Table 3). Apart from the "*Cylindrocarpon*"-like fungi, *Cadophora luteo-olivacea* was the species that occurred the most

and was present on 66 plant parts of the nursery stone fruit trees and on 16 plant parts of the ungrafted, rooted rootstock plants (Table 3). None of the fungal species were present in all of the sampling strategies. *Diplodia seriata* isolates were found in dormant scion buds, ungrafted rooted rootstock plants, dormant rootstock shoots and nursery plants, however, not in the green scion buds. Additional to possible pathogen candidates, a large variety of saprophytes species were isolated such as *Alternaria*, *Aureobasidium*, *Chaetomium*, *Epicoccum*, *Fusarium*, *Penicillium*, *Rhizopus* and *Trichoderma*.

Scion material

A total of 0.4% of the green scion buds were infected with canker pathogens with one bud infected with "*Cylindrocarpon*"-like fungi. The null hypothesis testing whether cultivar had an effect on fungal incidence was rejected (P = 0.0017) and differences were observed between infection percentages of the different scion cultivars. The one nectarine scion cultivar (Nectarine 1) had higher infections compared to the plum scion cultivars (Table 4). Six fungal species were identified from the green scion material with *Coniochaeta prunicola* (7 buds) being the species that infected the highest number of buds followed by *Biscogniauxia* sp. (3 buds). Only *Coniochaeta velutina* and *Thelonectria* sp. 2 were also isolated from the nursery stone fruit trees (Table 3).

For the dormant scion material, a total of 1.18% of the buds were infected with canker pathogens. No differences were observed between the three plum cultivars as the null hypothesis was not rejected (P = 0.2781) (Table 4). Higher percentages of infection were seen in the plum dormant scion cultivars in comparison with the plum green scion cultivars. Six species were identified with *Truncatella angustata* (17 buds) and *Didymella pomorum* (11 buds) occurring the most. *Truncatella angustata* were also frequently isolated from the nursery trees (Table 3).

Rootstock material

Out of the total number of ungrafted, rooted rootstock plants (378), 40 plants (10.58%) were infected with canker and wood rot pathogens and 24 plants (6.35%) were infected with "*Cylindrocarpon*"-like fungi. Differences were observed between the rootstock cultivars (P <0.0001) which indicates that the infection was dependent on the cultivar (Table 5). The Plum 1 rootstock cultivar had the highest infection while the Nectarine 2 rootstock cultivar had a low infection percentage with only one plant infected (Table 5). A variety of canker, wood rot and "*Cylindrocarpon*"-like fungi infected the ungrafted, rooted rootstock plants with a total of 22 species identified (Table 3). *Cadophora luteo-olivacea* was the species that infected the most plants (15) and was also the most frequently isolated from the nursery stone fruit trees. Most

of the species were isolated from the crown of the ungrafted, rooted rootstock plants and when the plant parts were cut open brown discolouration could be seen (Fig. 25).

A total of 6.17% of the dormant rootstock shoots were infected with canker pathogens and differences were observed between the different rootstock cultivars (P <0.0001) (Table 6). The plum rootstock cultivars (Plum 1, Plum 2 and Plum 3) had higher infection percentages than the nectarine rootstock cultivar (Nectarine 1) with Plum 3 having the most shoots infected (Table 6). Seven species were isolated from the dormant rootstock shoots with *Cytospora leucostoma* that infected the highest number of shoots (21), the majority from Plum 3 shoots (18 of the 19 infected shoots). Only *Didymosphaeria rubi-ulmifolii*, *Diplodia seriata* and *Truncatella angustata* were also isolated from the nursery stone fruit trees (Table 3).

Nursery trees

Out of the total number of nursery stone fruit trees sampled (1080 trees), there were 39.6% (428 trees) of the trees infected with canker, wood rot or "*Cylindrocarpon*"-like fungi. The "*Cylindrocarpon*"-like fungi infected a total of 255 trees (23.6%) and the canker and wood rot associated fungi infected a total of 235 trees (21.8%). These certified nursery trees did not show external disease symptoms, however, when cross sectioned brown discoloration from the pruning wound, bud union and crown were observed (Fig. 26). The trees that were infected with canker and wood rot pathogens per cultivar combination did not show significant differences (P = 0.3437) (Addendum B, Table 1). Nursery trees infection levels varied from 39.17% for Plum 3-1 to 3.33% for Nectarine 1-2. The trees that were infected with "*Cylindrocarpon*"-like fungi for the different cultivar combinations showed significant differences between cultivar combinations (P = 0.0087) (Addendum B, Table 2). The cultivar combination Plum 1-1 had higher infection with "*Cylindrocarpon*"-like fungi, however, it did not differ significantly from Plum 3-1 (Table 7). The combinations Nectarine 1-2 and Plum 3-2 had lower infection with "*Cylindrocarpon*"-like fungi, however, both combinations did not differ significantly from Plum 2-2, Plum 1-3,2-3,3-3 and Nectarine 1-1 (Table 7).

The different plant parts (crown, bud union and rootstock wound) infected with canker and wood rot pathogens did not differ from each other on a 5% level of significance, therefore in this case a 10% level of significance (P = 0.0596) (Addendum B, Table 3) was used to identify trends. The crown (11.13%) was significantly more infected in comparison with the wound on the rootstock (6.17%) (Table 8). For the "*Cylindrocarpon*"-like fungi infecting the different plant parts, a significant interaction was found with cultivar combination x plant part, therefore, the results are presented at the infection of plant parts level and were significant (P = <.0001) (Addendum B, Table 4). Infection with "*Cylindrocarpon*"-like fungi were significantly higher in the crown compared to the bud union and the wound (Table 8). Furthermore, at the crown significant differences were observed between the different cultivar combinations

infected with "*Cylindrocarpon*"-like fungi (*P* = 0.0026) (Addendum B, Table 5). Combination Plum 1-1 had higher infection in the crown with "*Cylindrocarpon*"-like fungi, however, it did not differ significantly from Plum 3-1 (Table 9). Lower infections with "*Cylindrocarpon*"-like fungi in the crown were observed in Nectarine 1-2 and Plum 3-2, however, both did not differ significantly from Plum 2-2, Plum 1-3,2-3,3-3 and Nectarine 1-1 (Table 9).

After the "*Cylindrocarpon*"-like fungi, *Cadophora* spp. (6.85%), Botryosphaeriaceae (4.72%) and Amphisphaeriaceae (2.96%) had the highest percentage latent infection in the nursery trees (Table 10). The distribution of canker and wood rot pathogens in different plants parts were investigated for the three most abundant canker fungal groups namely: Amphisphaeriaceae, Botryosphaeriaceae and *Cadophora* spp. (Table 11). Significant differences were observed on a plant part x taxonomic group level (*P* = 0.0026) (Addendum B, Table 6). The infection with Amphisphaeriaceae was higher in the crown, however it did not differ significantly from the bud union or wound. The Botryosphaeriaceae had higher infection in the bud union, however it did not differ significantly from the wound and crown. Only for *Cadophora* spp. significant differences were observed between the plant parts infected, with the crown having a higher level of infection versus the bud union and the wound.

Comparing tissue culture rootstock trees and hardwood rootstock trees for canker and wood rot pathogen infection, no significant differences were observed between the two propagation types for the crown, bud union or wound (data not shown). For the infection of the tissue culture rootstock trees and hardwood rootstock trees with "*Cylindrocarpon*"-like fungi, the null hypothesis was rejected at a 90% confidence level for the crown (*P* = 0.0765) (Table 12). Hardwood plants had higher infection levels in the crown (Table 12). The fungal organisms that were isolated from the hard wood rootstock trees were mostly "*Cylindrocarpon*"-like fungi (Table 13). From the tissue culture rootstock trees mostly canker associated fungi were isolated with *D. seriata* more often found (Table 13).

Pathogenicity test

Significant differences were found between the mean lesion lengths of the isolates of Orchard 1 (*P* = < 0.0001) (Addendum B, Table 7) and Orchard 2 (*P* = < 0.0001) (Addendum B, Table 8). Therefore, the mean lesion lengths per isolate is given separately for Orchard 1 (Table 14) and Orchard 2 (Table 15). All 66 isolates formed significantly longer lesions than the controls for both Orchard 1 (Table 14) and Orchard 2 (Table 15). Variation in the mean lesion lengths ranged from 19.59 mm to 229.94 mm for Orchard 1 and 22.60 mm to 191.98 mm for Orchard 2. Brown to black lesions were seen, typical of canker pathogens (Figs. 27-29). The longest lesions in Orchard 1 were formed by *Lasiodiplodia theobromae* isolates (STEU 8849 and STEU 8850), significantly different from all the other isolates. In Orchard 2, both *Lasiodiplodia theobromae* isolates (STEU 8849 and STEU 8850) also formed the longest lesions, however,

only isolate STEU 8850 was significantly different from the rest of the isolates. Isolate STEU 8849 did not form lesions that were significantly longer than *Biscogniauxia mediterranea*, which formed the third longest lesion. All the isolates included in the test were re-isolated from the inoculated shoots for both of the orchards, while no fungal pathogens were isolated from the controls. The re-isolation percentages varied from 5% to 89% for Orchard 1 (Table 14) and 21% to 100% for Orchard 2 (Table 15). The highest re-isolation percentage were for *Pleurostoma richardsiae* for both orchards, with 89% (isolate STEU 8937) for Orchard 1 (Table 14) and 100% (isolate STEU 8936) for Orchard 2 (Table 15), while the lowest re-isolation percentage were for *Eutypella* sp. from both orchards, with 5% for Orchard 1 (Table 14) and 21% for Orchard 2 (Table 15).

DISCUSSION

This study revealed that stone fruit nursery trees can have latent infection of canker and wood rot pathogens. Of the 1080 trees, 21.8% were infected with fungal species associated with canker and wood rot. Certified nursery trees are not tested for latent fungal infections and these infections could lead to losses in newly established stone fruit orchards (Van Rensburg, 1997; Mostert *et al*., 2016b). Infected trees start expressing symptoms when the trees experience stress conditions and can cause dieback and death of the young trees (Slippers and Wingfield, 2007).

Fifty-five fungal species associated with canker or wood rot were isolated in this study from propagation material (scion and rootstock) and nursery trees. Nineteen have been reported on stone fruit trees in South Africa and 26 are first reports on stone fruit trees in South Africa, which include *Biscogniauxia mediterranea, Cadophora gregata*, *Cadophora luteo-olivacea*, *Cadophora malorum*, *Cadophora novi-eboraci*, *Cadophora spadicis, Coniochaeta hoffmannii*, *Coprinellus flocculosus*, *Coprinellus micaceus*, *Cytospora austromontana*, *Diaporthe aspalathi*, *Diaporthe foeniculina*, *Didymella americana*, *Didymella pomorum*, *Dothiorella moneti*, *Eutypa leptoplaca*, *Lasiodiplodia theobromae*, *Neopestalotiopsis javaensis*, *Paraphaeosphaeria neglecta*, *Paraphaeosphaeria sporulosa*, *Paraphoma chrysanthemicola*, *Paraphoma radicina*, *Pleurostoma richardsiae*, *Truncatella angustata*, *Truncatella restionacearum* and *Valsa sordida*. Ten putative new species were found which include *Biscogniauxia* sp., *Cadophora* sp. 1, *Cadophora* sp. 2, *Coniochaeta* sp. 1, *Coniochaeta* sp. 2, *Coniochaeta* sp. 3, *Cytospora* sp. 1, *Cytospora* sp. 2, *Eutypella* sp. and *Peniophora* sp. Four species have been reported as canker or wood rot pathogens on stone fruit trees in other countries, but not yet in South Africa. *Lasiodiplodia theobromae* has been reported on plum (Inderbitzin *et al*., 2010) and almond (Chen *et al*., 2013) trees in California and peach trees in China and Turkey (Wang *et al*., 2011; Endes *et al*., 2016). *Diaporthe foeniculina* has been reported on almond trees in Portugal, California and Italy (Diogo *et al*.,

2010; Chen *et al*., 2014; Santos *et al*., 2017). *Eutypa leptoplaca* has been reported on plum trees in Argentina (Carmarán *et al*., 2009) and *Paraphoma radicina* on cherries in Australia (de Gruyter *et al*., 2010). Thus, 22 species have been recorded for the first time on stone fruit trees worldwide. The 10 putative new species needs to be described. All of the 38 species included in the pathogenicity trial formed significant lesions confirming their canker pathogen status on plum trees.

Biscogniauxia mediterranea is a known pathogen causing charcoal canker on forest trees and associated with wood decay of oak trees (Raimondo *et al*., 2016). A species not found in the current study, *Biscogniauxia rosacearum* M.L. Raimondo & Carlucci has been isolated from pear, plum and quince trees in Italy by Raimondo *et al*. (2016). However, in this study only *Biscogniauxia mediterranea* and an unknown *Biscogniauxia* species were isolated from stone fruit trees. Some of the Botryosphaeriaceae species isolated in this study have been identified from stone fruit trees in South Africa by Damm *et al*. (2007a) and are known to cause dieback and cankers on woody hosts (Slippers *et al*., 2007; Mehl *et al*., 2013; Sessa *et al*., 2016). The Tympanidaceae and Coniochaetaceae isolated in this study were also isolated from stone fruit trees in South Africa from wood necroses symptoms (Damm *et al*., 2010). Species in the Valsaceae that were isolated in this study are causal agents of Cytospora canker, also called Leucostoma canker on stone fruit trees as described by Ogawa *et al*. (1995). Some of the Diaporthaceae isolated in this study have also been isolated from stone fruit trees, where it caused cankers and dieback (Smit *et al*., 1996; Lawrence *et al*., 2015; Santos *et al*., 2017). The species of Diatrypaceae are important pathogens of grapevines (Trouillas *et al*., 2010), however a recent study isolated some of the Diatrypaceae species from symptomatic stone fruit trees (Moyo *et al*., 2018). Species of the Didymosphaeriaceae were isolated from stone fruit trees in this study as well as in the study by Damm *et al*. (2008c) and from apple (Havenga, 2017) and pear trees (Cloete *et al*., 2011) in South Africa where it caused, among other, symptoms of wood necroses and cankers. The *Neophaeomoniella* species isolated in this study have previously been isolated from necrotic wood of stone fruit trees in South Africa by Damm *et al*. (2010). Species isolated in this study within the Togniniaceae are known pathogens on stone fruit trees as the same *Phaeoacremonium* species were isolated by Damm *et al*. (2008b) from stone fruit trees showing symptoms of wood necroses.

Basidiomycetes are known pathogens causing wood rot of fruit trees and some species have been reported in South Africa to cause wood rot on stone fruit trees (Matthee and Thomas, 1977a). Species of the Basidiomycetes isolated in this study are pathogens associated with wood rot. Only *Schizophyllum commune* was previously reported from stone fruit trees (Crous *et al*., 2000). *Coprinellus* species have been reported to cause decay of

poplar wood (Olivier, 2008) and *Peniophora* species have been reported to cause wood rot and decay of pine and gum trees (Van der Westhuizen, 1972).

Some species identified in this study are known pathogens, however, some have not been reported as pathogens of stone fruit. Species within the Amphisphaeriaceae were identified as causal agents of grapevine trunk diseases and were the second most isolated group from cankers on grapevines in Texas (Urbez-Torres *et al*., 2011). The *Cadophora* species isolated from the stone fruit propagation material and nursery trees are not known pathogens on stone fruit, however, it has been reported to cause wood decay and decline of grapevines (Halleen *et al*., 2007; Gramaje *et al*., 2011; Travadon *et al*., 2015). Species of the Didymellaceae are less known canker pathogens, however it has been reported to cause stem necroses on black mulberry plants in Iran (Ahmadpour *et al*., 2017). Species of the Phaeosphaeriaceae, within the genus *Paraphoma*, which is a section within the *Phoma* (de Gruyter *et al*., 2010), have been isolated in this study and some species are known from decayed wood while others can invade weak plant tissue as secondary invaders (Aveskamp *et al*., 2008). The Calosphaeriaceae species isolated in this study are from the genus *Pleurostoma* which is associated with trunk diseases of grapevines (Halleen *et al*., 2007; Carlucci *et al*., 2015).

Of the nursery trees, 23.6% had infections with "*Cylindrocarpon*"-like species. None of the 14 "*Cylindrocarpon*"-like species have been reported on stone fruit trees in South Africa. Only *Ilyonectria robusta* have been reported on cherries in Canada (Cabral *et al*., 2012). The relevance of these species need to be established with pathogenicity trials. Of the different fungal groups, "*Cylindrocarpon*"-like fungi were isolated more frequently. These fungi together with a complex of other organisms which include *Thielaviopsis basicola* (Berk. & Broome) Ferraris, *Pythium* spp., *Phytophthora* spp., *Fusarium* spp., *Armillaria mellea* (Vahl.:Fr.) P. Kumm., *Clitocybe tabescens* Bres. and *Peniophora sacrata* G. Cunn together with biotic and abiotic interactions, are associated with replant disorder on stone fruit (Ogawa *et al*., 1995). However, replant disorder has not been reported on stone fruit trees in South Africa. The main fungal species associated with apple replant disease in South Africa were identified as *Ilyonectria destructans* (Zinssm.) Rossman, L. Lombard & Crous, *Ilyonectria liriodendri*, *Dactylonectria macrodidyma* and *Dactylonectria pauciseptatum* (Schroers & Crous) L. Lombard & Crous (Tewoldemedhin *et al*., 2011). Some *Thelonectria* species were reported by Salgado-Salazar *et al*. (2012; 2015) to be associated with small cankers on shrubs and trees of *Rubus* species (berries). Species belonging to the genera *Cylindrodendrum*, *Dactylonectria* and *Ilyonectria* were reported to cause loquat decline in Spain with symptoms of rotted roots observed (Agustí-Brisach *et al*., 2016). In this study, the same species associated with apple replant disease, cankers on *Rubus* species and loquat decline have been isolated from stone

fruit trees, showing the importance of understanding the role of "*Cylindrocarpon*"-like fungi in decline of young stone fruit trees.

The "*Cylindrocarpon*"-like fungi were isolated from the crown of the nursery trees mainly. This group of fungi is soil borne and associated with symptoms that include root rots with necrotic sunken lesions, stem cankers and necrotic xylem tissue at the base of the plant on a various range of woody hosts (Mai and Abawi, 1981; Halleen *et al*., 2004; Tewoldemedhin *et al*., 2011; Lombard *et al*., 2013; Agustí-Brisach *et al*., 2016; Carlucci *et al*., 2017). A study was done on peach seedlings in California showing the severe damage caused by a complex which include "*Cylindrocarpon*"-like fungi (Bent *et al*., 2009). The seedlings showed a reduction in growth of the plant and a reduction in the yield when exposed to as little as 1% of infected soil (Bent *et al*., 2009). The soil nutrition and structure can be abiotic factors playing a role in the disease expression together with roots remaining from crops previously planted in the soil, acting as support for microorganisms surviving in the soil until it can colonize in the next host (Gur and Cohen, 1989; Halleen *et al*., 2004). Very little is known about "*Cylindrocarpon*"-like fungi on stone fruit trees, raising the question if it is an economical problem for stone fruit growers.

Isolations from scion and rootstock material showed that these materials can be a source of latent infections for nursery trees. When comparing the species found in the scion and rootstock material to the species found in the nursery trees, 21 species were found in either the scion material or rootstock material as well as the nursery trees, while 7 species were only found in the scion material and 7 species only in the rootstock material. The fungal organisms isolated from the green scion material are mostly different in comparison to the fungal organisms isolated from the dormant scion material, with only *Biscogniauxia* species isolated from both green and dormant scion material. The time when scion and rootstock shoots are harvested is critical as spores of different fungal pathogens can be released more abundantly in different seasons (Bertrand and English, 1976; Pusey, 1989; Ogawa *et al*., 1995). Higher infection percentages were observed in the dormant scion material, which is harvested later than the green scion material. This could be due to a longer period of exposure to aerial inoculum as well as the onset of the rainy season which would increase aerial spore loads.

The dormant rootstock shoots harvested from the mother blocks already had infections before it was planted out in the field. Only canker pathogens were isolated from the dormant rootstock shoots, however, canker pathogens together with a number of "*Cylindrocarpon*"-like fungi were isolated from the ungrafted, rooted rootstock plants. The dormant rootstock shoots are planted with an open wound at the base and grow for one season before occulation. Most of the infection were observed from the crown of the ungrafted, rooted rootstock plants. Differences were observed between the rootstock cultivars with Nectarine 2 rootstock having

the lowest infection. This is due to the rootstock Nectarine 2 being a seedling variety, not having an open wound at the base.

In a study on apple nursery trees and propagation material, it was found that similar species were isolated from the scion and rootstock material as from the nursery trees (Havenga, 2017). In this study, the same trend was found for some of the most frequently isolated species. *Cadophora luteo-olivacea* was the most isolated species from the ungrafted, rooted rootstock plants and the canker pathogen isolated the most from the nursery trees. *Diplodia seriata* and *Truncatella angustata* were both isolated from the dormant scion buds, ungrafted, rooted rootstock plants, dormant rootstock shoots and nursery trees. Seven out of the 14 "*Cylindrocarpon*"-like species were isolated from both the ungrafted, rooted rootstock plants and the nursery trees. Therefore, infection of nursery trees with canker and wood rot pathogens and "*Cylindrocarpon*"-like fungi could originate from the propagation material used. Thirty of the species were isolated from the nursery trees only and not from the propagation material. This emphasise the importance of good sanitation practices in the nurseries and protection of wounds as well as the wound at the crown of the nursery trees. The wood rot fungi were not found on the scion and rootstock cuttings from the mother block trees, however it was found on the ungrafted, rooted rootstock plants and nursery trees.

The disease susceptibility of cultivars is influenced by their inherent resilience towards plant stress (Mehl *et al*., 2013; Anonymous, 2015). The different cultivar combinations of the nursery trees showed that the infection levels were different between the combinations infected with "Cylindrocarpon"-like fungi. The combination Plum 1-1 and Plum 3-1 had higher percentage trees infected with "*Cylindrocarpon*"-like fungi. In addition, Plum 1 rootstock cultivar had higher percentage infection of the ungrafted, rooted rootstock cultivars as well as the dormant rootstock cultivars. This indicates that Plum 1 rootstock is more susceptible to "*Cylindrocarpon*"-like fungi. For both canker and wood rot pathogens and "*Cylindrocarpon*"-like fungi, Nectarine 1-2 was the combination which had the least trees infected. Although scion cultivar Nectarine 1 had the highest number of infected buds, the rootstock cultivar Nectarine 2 were the seedling cultivar which resulted in the lowest infection percentages for the ungrafted, rooted rootstock cultivars, again showing that the seedling rootstock without the open wound at the base prevent infection from soil borne fungi. Seedling rootstocks are important for stone fruit production, however, in a study by Browne *et al*. (2013), different clonal rootstocks made with certain peach x almond, peach and plum hybrid selections showed promising results by having low sensitivity to the replant complex.

In this study, the infection of nursery trees made with tissue culture rootstock plants was compared to trees made with hardwood rootstock cuttings from rootstock mother block trees. The tissue culture trees had lower infection with "*Cylindrocarpon*"-like fungi in the crown, however, infection with canker and wood rot pathogens still occurred in the bud union and in

the rootstock wound. This shows the importance of sanitation practices during the occulation process and covering of the wound with an effective wound sealant (Matthee and Thomas, 1977b; Van Zyl, 2011). A more extensive study (more than 40 plants) is, however, needed to assess the advantages of tissue culture generated plant material.

Canker and wood rot pathogens were mostly isolated from the pruning wound on the rootstock and the bud union of apple nursery trees in a study by Havenga (2017). In contrast, this study found that most of the canker pathogens were isolated from the crown of the nursery trees. The reason for that being the abundance of *Cadophora* species in the crown of the ungrafted, rooted rootstock plants and nursery trees. In the study by Havenga (2017), *Cadophora* species were not found as often as in this study, most probably due to the rooted rootstocks used for nursery apple trees. Spores of Botryosphaeriaceae are found abundantly in the air, being a source of aerial inoculum which can land on open wounds during budding in the field and when rootstocks are cut back after budding (Van Niekerk *et al*., 2010). Infection by means of aerial inoculum can be a result of other infected orchards in close proximity to nursery orchards. Pome fruit orchards and vineyards are seen as alternative hosts for stone fruit pathogens and care should be taken that correct sanitation practices are applied to minimise these inoculum sources (Mostert *et al*., 2016a). In this study, Botryosphaeriaceae which was the second most isolated taxonomic group, was mostly isolated from the bud union, which was the second most infected plant part with canker and wood rot pathogens of the nursery trees. Damm *et al*. (2007a) also isolated Botryosphaeriaceae from stone fruit trees in South Africa and also found *Diplodia seriata* more frequently, as in this study.

Pathogenicity studies were conducted on plum trees for the canker and wood rot pathogens isolated in this study. The results for both orchards showed that both isolates of *Lasiodiplodia theobromae* were the most virulent. In Orchard 1, both isolates of *L. theobromae* differed significantly from all the other isolates, however, in Orchard 2 isolate STEU 8849 did not differ from *Biscogniauxia mediterranea*, but those two isolates were significantly different from all the other isolates. Both *L. theobromae* and *B. mediterranea* have not been reported from stone fruit in South Africa, however *L. theobromae* have been isolated from cankers on almonds in Spain (Chen *et al*., 2013) and dieback symptoms on nectarines in Turkey (Endes *et al*., 2016). Pathogenicity studies confirmed that *L. theobromae* is pathogenic to almonds and nectarines in a detached shoot assay by Chen *et al.* (2013) and a field trial by Endes *et al.* (2016). Interestingly, a study on grapevines in Texas found that *L. theobromae* is the most virulent species in comparison to ten other species isolated from cankers on grapevines (Úrbez-torres *et al*., 2009). A study in Mexico on grapevines also confirmed that *L. theobromae* is the more virulent species in comparison to *Diplodia seriata* (Urbez-Torres *et al*., 2008). This shows the importance of *L. theobromae* as a pathogen of stone fruit trees as results of this study also confirmed its pathogenicity status on plum trees. *Biscognauixia* species are well

known to cause charcoal canker on oak trees, furthermore, a study by Raimondo *et al*. (2016) in Southern Italy proved that *Biscogniauxia rosacearum* is pathogenic to plum. In addition, the current study confirmed that *Biscognauixia mediterranea* is indeed a pathogen on plum trees, being one of the more virulent species.

In studies done by Damm *et al*. (2007a; 2008b, c; 2010) on stone fruit trees, pathogenicity of some species were tested. Species of Botryosphaeriaceae were tested on green nectarine and plum shoots in a detached shoot assay assessed after two weeks (Damm *et al.*, 2007a). Results from the pathogenicity test showed that *Diplodia seriata* were pathogenic to plum and nectarine and *Dothiorella viticola* were considered as non-pathogenic to the hosts (Damm *et al.*, 2007a). In this study, *D. seriata* was shown to be pathogenic to both plum cultivars in the two orchard trials, however, in contrast with Damm *et al*. (2007a), *Dothiorella viticola* is considered to be pathogenic to plum according to the results of both orchards. Another study done by Damm *et al*. (2010) reported species of *Coniochaeta* and *Collophorina* among others, from necrotic wood of *Prunus* species. On a detached shoot assay of green apricot, peach and plum shoots assessed after two weeks, *Collophorina paarla* were considered to be pathogenic on plum only, *Collophorina rubra* was considered to be pathogenic on apricot only and *Coniochaeta velutina* was not found to be pathogenic on any of the three hosts. *Collophorina paarla* and *C. rubra* were found to be pathogenic to plum in both orchards in this study. In contrast with Damm *et al*. (2010), this study found that *Coniochaeta velutina* did make lesions which were significantly longer than the control. *Coniochaeta hoffmannii* has not previously been reported from stone fruit or any other host in South Africa (Farr and Rossman, 2018), however, results from the pathogenicity test showed that this species is pathogenic to plum. Two putative new *Coniochaeta* species isolated in this study, were pathogenic to plum.

Phaeoacremonium species are known from a wide range of hosts in South Africa (Spies *et al*., 2018) and pathogenicity studies were done on species isolated from *Prunus* trees by Damm *et al*. (2008b). *Phaeoacremonium parasiticum* and *Phaeoacremonium iranianum* were chosen to inoculate in the present study since these two species formed the longest lesions in the detached green plum and apricot shoots assessed after two weeks by Damm *et al*. (2008b). The results from the pathogenicity test confirmed that these two species are pathogenic to plum.

Three species of *Paraconiothyrium* have been isolated from stone fruit trees in South Africa, namely *Paraconiothyrium africanum* Damm, Verkley & Crous, *Didymosphaeria rubi-ulmifolii* (synonym *Paraconiothyrium brasiliense* Verkley) and *Didymosphaeria variabile* (synonym *Paraconiothyrium variablile* Riccioni, Damm, Verkley & Crous), but no pathogenicity tests have been done (Damm *et al.*, 2008c). In the current study, only *Didymosphaeria rubi-ulmifolii* and *Didymosphaeria variabile* were isolated. *Didymosphaeria variabile* were

considered pathogenic to plum, though in Orchard 2 the two isolates' mean lesion lengths were significantly different. Both isolates of *Didymosphaeria rubi-ulmifolii* were confirmed to be pathogenic to plum.

Isolates from the same species can differ in their virulence (Smit *et al*., 1996). In Orchard 1, *Diaporthe ambigua* (isolate STEU 8901 and STEU 8902), *Cytospora leucostoma* (isolates STEU 8881 and STEU 8882), *Truncatella angustata* (isolates STEU 8834 and STEU 8835) and *Cytospora* sp. 2 (isolates STEU 8886 and STEU 8887) formed lesions that were statistically different. Only for *Didymosphaeria variabile* (isolates STEU 8912 and STEU 8913) did the lesion size differ significantly between the two isolates tested in Orchard 2. This illustrate the variability of virulence between isolates of the same species.

In this study *Cadophora luteo-olivacea*, *Diplodia seriata* and *Truncatella angustata* were isolated the most from the stone fruit propagation material together with the nursery trees. The pathogenicity test confirmed that *Diplodia seriata* is an important pathogen on stone fruit which was also found in a previous study (Damm *et al*., 2007a). *Cadophora luteo-olivacea* and *Truncatella angustata* have not been reported from stone fruit worldwide. Both species are considered to be pathogenic to plum according to the results of this study. Pathogenicity tests of *Cadophora luteo-olivacea* on grapevine and apples also showed that this species can cause lesions on woody stems (Gramaje *et al*., 2011; Travadon *et al*., 2015; Gatsi, 2017). *Truncatella angustata* has been isolated from other hosts, like grapevine, where pathogenicity tests showed that *T. angustata* can cause symptoms of decline (Arzanlou *et al*., 2013; Maharachchikumbura *et al*., 2016). *Truncatella angustata* was also isolated from apple plant material and pathogenicity tests on field grown apple trees proved it to be pathogenic (unpublished data). The current study also proved that *Truncatella angustata* can be considered as a pathogen of plum.

Various fungal groups associated with canker and wood rot have been identified from diseased stone fruit trees by the Disease Clinic of Stellenbosch University. Species within the Botryosphaeriaceae was mostly found by the Disease Clinic and in this study specifically for the canker and wood rot pathogens, after the *Cadophora* species, the Botryosphaeriaceae species were also frequently isolated as canker pathogens from stone fruit propagation material and nursery trees. Some of the lesser known genera on stone fruit found in this study such as species of *Truncatella*, *Paraphoma* and *Biscogniauxia* could accidently have been thrown out by the Disease Clinic as unknown fungi. The growth of species after 7 days and 14 days on PDA was captured for all the species included in the pathogenicity trial in this study to aid in future identification of the canker and wood rot species (Addendum C, Figs. 1-7).

Management of canker and wood rot pathogens present on stone fruit nursery trees as well as the propagation material used, should be implemented. By reducing abiotic and biotic stress factors on trees during the propagation process, storage and when trees are

established in new orchards could reduce the occurrence of diseases on the trees. Factors such as the water and nutrient availability and damage caused by other insects and diseases and incorrect cultural practices should be avoided to lower stress on the young trees (Steyn *et al.*, 2016). Aerial inoculum should be reduced by implementing good sanitation practices in orchards and the surrounding areas. Branches, twigs and any material infected with canker or wood rot pathogens should be removed and discarded by burning (Van Zyl, 2011). Especially in nursery orchards where rootstock shoots are planted, the rootstocks which did not grow should be removed from the orchards before the budding process as it can act as a source of inoculum.

Havenga (2017) found that younger apple scion mother block orchards were less infected than the older mother block orchards. This suggest that the scion and rootstock mother block orchards used for propagation material should be renewed more often. When scion and rootstock propagation material are harvested, a lot of wounds are made at the same time on the mother trees which create infection points for aerial inoculum of canker and wood rot pathogens. Plant improvement organisations should consider applying a pruning wound sealant containing a fungicide to minimise infections in mother block orchards (Matthee and Thomas, 1977b). Also, pruning wound protection should be used in stone fruit nurseries when the rootstock is cut back in spring.

Canker and wood rot pathogens were found in nursery trees which was distributed to producers as seemingly healthy, certified nursery trees. The stone fruit nursery trees could get infected by means of aerial inoculum or from infected scion and rootstock propagation material. Pathogenicity tests revealed that the canker and wood rot pathogens tested in this study are pathogenic to plum trees. Additional pathogenicity tests should be conducted for the "*Cylindrocarpon*"-like fungi on stone fruit rootstock trees. Future work should aim at finding the age limit of mother block orchards to ensure less pathogen infected scion and rootstock material. Sanitation practices should be improved during the propagation process as well as in the nursery tree orchards. Lastly, pruning wound protectants should be tested on stone fruit nursery trees and on scion and rootstock mother block orchards.

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TABLES AND FIGURES

Table 1. Nursery tree scion and rootstock combinations, collected from three different nurseries in South Africa.

Scion cultivar	Rootstock cultivar	Number of trees
Nectarine 1	Nectarine 1	120
	Nectarine 2	120
Plum 1	Plum 1	120
	Plum 2	120
	Plum 3	40
Plum 2	Plum 1	120
	Plum 2	120
	Plum 3	40
Plum 3	Plum 1	120
	Plum 2	120
	Plum 3	40

Table 2. Species taxonomic and isolation details of representative cultures for all the species reported from stone fruit propagation material and nursery trees

Strain number	Taxonomic group	Fungal taxa	Plant part	Host
STEU 8833	Amphisphaeriaceae	*Neopestalotiopsis javaensis*	Bud union of a nursery tree	Almond
STEU 8834		*Truncatella angustata*	Bud union of a nursery tree	Plum
STEU 8835		*Truncatella angustata*	Crown of a nursery tree	Nectarine
STEU 8836		*Truncatella restionacearum*	Dormant scion bud	Plum
STEU 8837	Basidiomycetes	*Coprinellus flocculosus*	Crown of ungrafted, rooted rootstock plant	Plum
STEU 8838		*Coprinellus micaceus*	Wound, crown and bud union of nursery trees	Plum
STEU 8839		*Coprinellus micaceus*	Crown of ungrafted, rooted rootstock plant	Plum
STEU 8840		*Peniophora* sp.	Wound of a nursery tree	Plum
STEU 8841		*Schizophyllum commune*	Wound of a nursery tree	Plum
STEU 8845	Botryosphaeriaceae	*Diplodia seriata*	Dormant rootstock shoot	Plum
STEU 8846		*Diplodia seriata*	Dormant rootstock shoot	Plum
STEU 8847		*Dothiorella moneti*	Dormant rootstock shoot	Plum
STEU 8848		*Dothiorella viticola*	Dormant scion bud	Plum
STEU 8849		*Lasiodiplodia theobromae*	Bud union of a nursery tree	Nectarine
STEU 8850		*Lasiodiplodia theobromae*	Bud union of a nursery tree	Nectarine
STEU 8851		*Neofusicoccum australe*	Wound of a nursery tree	Plum
STEU 8852		*Neofusicoccum australe*	Bud union of a nursery tree	Plum
STEU 8854	*Cadophora* spp.	*Cadophora gregata*	Crown and bud union of nursery trees	Plum
STEU 8855		*Cadophora gregata*	Crown of a nursery tree	Plum
STEU 8856		*Cadophora luteo-olivacea*	Crown of ungrafted, rooted rootstock plant	Plum
STEU 8857		*Cadophora luteo-olivacea*	Crown of a nursery tree	Plum
STEU 8858		*Cadophora malorum*	Crown of a nursery tree	Plum
STEU 8859		*Cadophora malorum*	Tip and crown of ungrafted, rooted rootstock plants	Plum
STEU 8860		*Cadophora novi-eboraci*	Wound of a nursery tree	Plum

Table 2. Continue.

Strain number	Taxonomic group	Fungal taxa	Plant part	Host
STEU 8861		*Cadophora spadicis*	Crown of a nursery tree	Plum
STEU 8862		*Cadophora* sp. 1	Crown of a nursery tree	Nectarine
STEU 8863		*Cadophora* sp. 2	Crown of a nursery tree	Plum
STEU 8864		*Cadophora* sp. 2	Crown of a nursery tree	Plum
STEU 8865		*Cadophora* sp. 2	Crown of a nursery tree	Plum
STEU 8936	Calosphaeriaceae	*Pleurostoma richardsiae*	Crown of a nursery tree	Nectarine
STEU 8937		*Pleurostoma richardsiae*	Bud union of a nursery tree	Plum
STEU 8869	Coniochaetaceae	*Coniochaeta hoffmannii*	Bud union of a nursery tree	Plum
STEU 8870		*Coniochaeta hoffmannii*	Crown of a nursery tree	Plum
STEU 8871		*Coniochaeta prunicola*	Green scion bud	Nectarine
STEU 8872		*Coniochaeta prunicola*	Green scion bud	Nectarine
STEU 8873		*Coniochaeta* sp. 1	Green scion bud	Plum
STEU 8874		*Coniochaeta* sp. 2	Crown of a nursery tree	Plum
STEU 8875		*Coniochaeta* sp. 2	Crown of a nursery tree	Plum
STEU 8876		*Coniochaeta* sp. 2	Crown of a nursery tree	Nectarine
STEU 8877		*Coniochaeta* sp. 3	Green scion bud	Plum
STEU 8878		*Coniochaeta velutina*	Crown of a nursery tree	Plum
STEU 8879		*Coniochaeta velutina*	Wound of a nursery tree	Plum
STEU 8853	"*Cylindrocarpon*"-like fungi	*Campylocarpon pseudofasciculare*	Crown and bud union of nursery trees	Plum
STEU 8890		*Dactylonectria macrodidyma*	Crown of ungrafted, rooted rootstock plant	Plum
STEU 8891		*Dactylonectria macrodidyma*	Crown of a nursery tree	Plum
STEU 8892		*Dactylonectria novozelandica*	Crown of ungrafted, rooted rootstock plant	Plum
STEU 8893		*Dactylonectria novozelandica*	Crown of a nursery tree	Plum
STEU 8894		*Dactylonectria* sp. 1	Crown of ungrafted, rooted rootstock plant	Plum

Table 2. Continue.

Strain number	Taxonomic group	Fungal taxa	Plant part	Host
STEU 8895		*Dactylonectria* sp. 1	Wound of a nursery tree	Plum
STEU 8896		*Dactylonectria* sp. 1	Crown of a nursery tree	Plum
STEU 8897		*Dactylonectria* sp. 2	Crown of a nursery tree	Plum
STEU 8898		*Dactylonectria* sp. 3	Crown and wound of nursery trees	Plum
STEU 8899		*Dactylonectria torresensis*	Crown of a nursery tree	Plum
STEU 8900		*Dactylonectria torresensis*	Crown of a nursery tree	Nectarine
STEU 8914		*Ilyonectria liriodendri*	Crown of a nursery tree	Plum
STEU 8915		*Ilyonectria liriodendri*	Crown and bud union of nursery trees	Plum
STEU 8916		*Ilyonectria robusta*	Crown of a nursery tree	Plum
STEU 8917		*Ilyonectria robusta*	Crown of a nursery tree	Plum
STEU 8918		*Ilyonectria* sp.	Crown of a nursery tree	Nectarine
STEU 8938		*Thelonectria* sp. 1	Crown of a nursery tree	Plum
STEU 8939		*Thelonectria* sp. 1	Crown of a nursery tree	Plum
STEU 8940		*Thelonectria* sp. 2	Green scion bud	Nectarine
STEU 8941		*Thelonectria* sp. 2	Crown of a nursery tree	Plum
STEU 8942		*Thelonectria truncata*	Crown of a nursery tree	Almond
STEU 8943		*Thelonectria truncata*	Crown of ungrafted, rooted rootstock plant	Nectarine
STEU 8944		*Thelonectria veuillotiana*	Crown of ungrafted, rooted rootstock plant	Plum
STEU 8945		*Thelonectria veuillotiana*	Crown of a nursery tree	Nectarine
STEU 8901	Diaporthaceae	*Diaporthe ambigua*	Bud union of a nursery tree	Plum
STEU 8902		*Diaporthe ambigua*	Tip of ungrafted, rooted rootstock plant	Plum
STEU 8903		*Diaporthe aspalathi*	Wound of ungrafted, rooted rootstock plant	Nectarine
STEU 8904		*Diaporthe foeniculina*	Wound of a nursery tree	Nectarine
STEU 8905	Diatrypaceae	*Eutypa leptoplaca*	Wound of a nursery tree	Plum
STEU 8906		*Eutypella* sp.	Bud union and wound of nursery trees	Plum

Table 2. Continue.

Strain number	Taxonomic group	Fungal taxa	Plant part	Host
STEU 8907	Didymellaceae	*Didymella americana*	Dormant rootstock shoot	Plum
STEU 8908		*Didymella pomorum*	Dormant rootstock shoot	Plum
STEU 8909		*Didymella pomorum*	Dormant scion bud	Plum
STEU 8910	Didymosphaeriaceae	*Didymosphaeria rubi-ulmifolii* s.s.	Wound of a nursery tree	Plum
STEU 8911		*Didymosphaeria rubi-ulmifolii* s.s.	Bud union of a nursery tree	Plum
STEU 8912		*Didymosphaeria variabile*	Bud union of a nursery tree	Plum
STEU 8913		*Didymosphaeria variabile*	Wound of a nursery tree	Nectarine
STEU 8920		*Paraphaeosphaeria neglecta*	Crown of a nursery tree	Plum
STEU 8921		*Paraphaeosphaeria neglecta*	Tip of ungrafted, rooted rootstock plant	Plum
STEU 8922		*Paraphaeosphaeria sporulosa*	Crown of a nursery tree	Plum
STEU 8923		*Paraphaeosphaeria sporulosa*	Wound of a nursery tree	Plum
STEU 8919	*Neophaeomoniella* sp.	*Neophaeomoniella zymoides*	Wound of a nursery tree	Plum
STEU 8924	Phaeosphaeriaceae	*Paraphoma chrysanthemicola*	Crown of a nursery tree	Plum
STEU 8925		*Paraphoma chrysanthemicola*	Crown of ungrafted, rooted rootstock plant	Plum
STEU 8926		*Paraphoma radicina*	Crown of ungrafted, rooted rootstock plant	Plum
STEU 8927	Togniniaceae	*Phaeoacremonium australiense*	Crown of a nursery tree	Plum
STEU 8928		*Phaeoacremonium fraxinopennsylvanicum*	Crown of a nursery tree	Plum
STEU 8929		*Phaeoacremonium iranianum*	Crown of a nursery tree	Plum
STEU 8930		*Phaeoacremonium iranianum*	Crown of a nursery tree	Plum
STEU 8931		*Phaeoacremonium minimum*	Bud union of a nursery tree	Plum
STEU 8932		*Phaeoacremonium minimum*	Crown of a nursery tree	Plum
STEU 8933		*Phaeoacremonium parasiticum*	Crown of a nursery tree	Plum
STEU 8934		*Phaeoacremonium parasiticum*	Bud union of a nursery tree	Plum
STEU 8935		*Phaeoacremonium scolyti*	Crown of a nursery tree	Plum

Table 2. Continue.

Strain number	Taxonomic group	Fungal taxa	Plant part	Host
STEU 8866	Tympanidaceae	*Collophorina paarla*	Tip of ungrafted, rooted rootstock plant	Plum
STEU 8867		*Collophorina paarla*	Bud union of a nursery tree	Plum
STEU 8868		*Collophorina rubra*	Tip and crown of ungrafted, rooted rootstock plants	Nectarine
STEU 8880	Valsaceae	*Cytospora austromontana*	Wound of a nursery tree	Nectarine
STEU 8881		*Cytospora leucostoma*	Dormant rootstock shoot	Plum
STEU 8882		*Cytospora leucostoma*	Dormant rootstock shoot	Plum
STEU 8883		*Cytospora* sp. 1	Wound of a nursery tree	Plum
STEU 8884		*Cytospora* sp. 1	Bud union of a nursery tree	Plum
STEU 8885		*Cytospora* sp. 1	Crown of a nursery tree	Plum
STEU 8886		*Cytospora* sp. 2	Wound of a nursery tree	Plum
STEU 8887		*Cytospora* sp. 2	Bud union of a nursery tree	Plum
STEU 8888		*Valsa sordida*	Bud union and wound of a nursery tree	Nectarine
STEU 8889		*Valsa sordida*	Tip of ungrafted, rooted rootstock plant	Plum
STEU 8842	Xylariaceae	*Biscogniauxia mediterranea*	Dormant scion bud	Plum
STEU 8843		*Biscogniauxia* sp.	Green scion bud	Plum
STEU 8844		*Biscogniauxia* sp.	Green scion bud	Plum

Table 3. Incidence of fungal taxa isolated from scion and rootstock stone fruit propagation material and nursery stone fruit trees.

Taxonomic group	Fungal organism [*]	Overall incidence [a]	Green scion buds [b]	Dormant scion buds [b]	Ungrafted, rooted rootstock plants [c]	Dormant rootstock shoots [d]	Nursery trees [e]
					Incidence for specific sampling strategy		
Amphisphaeriaceae	*Neopestalotiopsis javaensis*	1	0	0	0	0	1
	Truncatella angustata	60	0	17	4	6	33
	Truncatella restionacearum	1	0	1	0	0	0
Basidiomycetes	*Coprinellus flocculosus*	1	0	0	1	0	0
	Coprinellus micaceus	16	0	0	1	0	15
	Peniophora sp.	1	0	0	0	0	1
	Schizophyllum commune [1,2]	1	0	0	0	0	1
Botryosphaeriaceae	*Diplodia seriata* [1,2]	65	0	3	3	4	55
	Dothiorella moneti	1	0	0	0	1	0
	Dothiorella viticola [1]	1	0	1	0	0	0
	Lasiodiplodia theobromae [2]	4	0	0	0	0	4
	Neofusicoccum australe [1]	3	0	0	0	0	3
Cadophora spp.	*Cadophora gregata*	11	0	0	5	0	6
	Cadophora luteo-olivacea	81	0	0	15	0	66
	Cadophora malorum	5	0	0	3	0	2
	Cadophora novi-eboraci	1	0	0	0	0	1
	Cadophora spadicis	1	0	0	0	0	1
	Cadophora sp. 1	1	0	0	0	0	1
	Cadophora sp. 2	3	0	0	0	0	3
Calosphaeriaceae	*Pleurostoma richardsiae*	7	0	0	0	0	7

Table 3. Continue.

			Incidence for specific sampling strategy				
Taxonomic group	**Fungal organism** [*]	**Overall incidence** [a]	**Green scion buds** [b]	**Dormant scion buds** [b]	**Ungrafted, rooted rootstock plants** [c]	**Dormant rootstock shoots** [d]	**Nursery trees** [e]
Coniochaetaceae	*Coniochaeta hoffmannii*	6	0	0	0	0	6
	Coniochaeta prunicola [1]	7	7	0	0	0	0
	Coniochaeta sp. 1	2	2	0	0	0	0
	Coniochaeta sp. 2	5	0	0	0	0	5
	Coniochaeta sp. 3	1	1	0	0	0	0
	Coniochaeta velutina [1]	13	1	0	0	0	12
"*Cylindrocarpon*"-like fungi	*Campylocarpon pseudofasciculare*	1	0	0	0	0	1
	Dactylonectria macrodidyma	65	0	0	6	0	59
	Dactylonectria novozelandica	90	0	0	3	0	87
	Dactylonectria sp. 1	35	0	0	1	0	34
	Dactylonectria sp. 2	2	0	0	0	0	2
	Dactylonectria sp. 3	21	0	0	0	0	21
	Dactylonectria torresensis	79	0	0	6	0	73
	Ilyonectria liriodendri	28	0	0	3	0	25
	Ilyonectria robusta [2]	6	0	0	0	0	6
	Ilyonectria sp.	1	0	0	0	0	1
	Thelonectria sp. 1	3	0	0	0	0	3
	Thelonectria sp. 2	9	1	0	0	0	8
	Thelonectria truncata	17	0	0	1	0	16
	Thelonectria veuillotiana	15	0	0	4	0	11
Diaporthaceae	*Diaporthe ambigua* [1,2]	3	0	0	1	0	2

Table 3. Continue.

Taxonomic group	Fungal organism [*]	Overall incidence [a]	Green scion buds [b]	Dormant scion buds [b]	Ungrafted, rooted rootstock plants [c]	Dormant rootstock shoots [d]	Nursery trees [e]
	Diaporthe aspalathi	1	0	0	1	0	0
	Diaporthe foeniculina [2]	1	0	0	0	0	1
Diatrypaceae	*Eutypa leptoplaca* [2]	1	0	0	0	0	1
	Eutypella sp.	4	0	0	0	0	4
Didymellaceae	*Didymella americana*	1	0	0	0	1	0
	Didymella pomorum	14	0	11	0	3	0
Didymosphaeriaceae	*Didymosphaeria rubi-ulmifolii* [1]	10	0	0	0	1	9
	Didymosphaeria variabile [1]	3	0	0	0	0	3
	Paraphaeosphaeria neglecta	3	0	0	1	0	2
	Paraphaeosphaeria sporulosa	11	0	0	0	0	11
Neophaeomoniella sp.	*Neophaeomoniella zymoides* [1]	1	0	0	0	0	1
Phaeosphaeriaceae	*Paraphoma chrysanthemicola*	4	0	0	1	0	3
	Paraphoma radicina [2]	1	0	0	1	0	0
Togniniaceae	*Phaeoacremonium australiense* [1,2]	1	0	0	0	0	1
	Phaeoacremonium fraxinopennsylvanicum [1]	1	0	0	0	0	1
	Phaeoacremonium iranianum [1,2]	1	0	0	0	0	1
	Phaeoacremonium minimum [1,2]	3	0	0	0	0	3
	Phaeoacremonium parasiticum [1,2]	3	0	0	0	0	3
	Phaeoacremonium scolyti [1]	1	0	0	0	0	1
Tympanidaceae	*Collophorina paarla* [1]	21	0	0	4	0	17
	Collophorina rubra [1]	2	0	0	2	0	0
Valsaceae	*Cytospora austromontana*	1	0	0	0	0	1

Table 3. Continue.

Taxonomic group	Fungal organism [*]	Overall incidence [a]	Green scion buds [b]	Dormant scion buds [b]	Ungrafted, rooted rootstock plants [c]	Dormant rootstock shoots [d]	Nursery trees [e]
	Cytospora leucostoma [1,2]	21	0	0	0	21	0
	Cytospora sp. 1	4	0	0	0	0	4
	Cytospora sp. 2	2	0	0	0	0	2
	Valsa sordida	11	0	0	1	0	10
Xylariaceae	*Biscogniauxia mediterranea*	1	0	1	0	0	0
	Biscogniauxia sp.	3	3	0	0	0	0

[*] Fungal species reported on stone fruit in South Africa ([1]) and on stone fruit in other countries ([2]) are indicated.

[a] Incidence is equal to the number of plant parts infected per organism.

[b] Incidence per bud out of a total of 3840 green buds and 2880 dormant buds.

[c] Incidence per plant out of a total of 378 plants.

[d] Incidence per shoot out of a total of 600 shoots.

[e] Incidence found in a total of 3240 plant parts of the nursery trees from either the rootstock wound, bud union or crown.

Table 4. Mean percentage of buds infected in the nectarine and plum scion cultivars.

Scion cultivar	Total buds	Infected green buds	Infected dormant buds	Infection of green buds(%) [a]	Infection of dormant buds(%) [b]
Nectarine 1	960	10	-	1.04	-
Plum 1	960	2	7	0.21	0.73
Plum 2	960	3	14	0.31	1.46
Plum 3	960	0	13	0.00	1.35

Probability value calculated with Chi-square under the null hypothesis that infected buds are independent of cultivar [a](P = 0.0017); [b](P = 0.2781).

Table 5. Mean percentage of nectarine and plum ungrafted, rooted rootstock plants infected.

Rootstock cultivar	Total plants	Infected plants	Infection (%)
Nectarine 1	80	11	13.75
Nectarine 2	80	1	1.25
Plum 1	80	25	31.25
Plum 2	98	19	19.39
Plum 3	40	7	17.50

Probability value calculated with Chi-square under the null hypothesis that infected plants are independent of cultivar (P < 0.0001).

Table 6. Mean percentage of nectarine and plum dormant rootstock shoots infected.

Rootstock cultivar	Total shoots	Infected shoots	Infection (%)
Nectarine 1	180	2	1.11
Plum 1	180	10	5.56
Plum 2	180	6	3.33
Plum 3	60	19	31.67

Probability value calculated with Chi-square under the null hypothesis that infected shoots are independent of cultivar (P < 0.0001).

Table 7. Mean percentage of nursery stone fruit trees infected with "*Cylindrocarpon*"-like fungi per cultivar combination.

Cultivar combination	Trees sampled	Infected trees (%) [a]
		"*Cylindrocarpon*"-like fungi
Plum 1-1 [b]	120	59.17 [A]
Plum 3-1	120	44.17 [AB]
Plum 2-1	120	30.83 [BC]
Plum 1-2	120	29.17 [BC]
Nectarine 1-1	120	22.50 [BCD]
Plum 1-3,2-3,3-3	120	14.17 [CD]
Plum 2-2	120	9.17 [CD]
Plum 3-2	120	2.50 [D]
Nectarine 1-2	120	0.83 [D]

[a] Means followed by the same letter do not differ significantly from each other at $P = 0.05$.

[b] First number in cultivar type refer to scion and second number to rootstock.

Table 8. Mean percentage infection with canker and wood rot pathogens and "*Cylindrocarpon*"-like fungi in the different plant parts isolated from the nursery stone fruit trees.

Plant part	Infection (%) [a]	
	Canker and wood rot pathogens[a]	"*Cylindrocarpon*"-like fungi[b]
Crown	11.13 [A]	21.25 [A]
Bud union	7.38 [AB]	1.21 [B]
Wound	6.17 [B]	1.25 [B]

[a] Means followed by the same letter do not differ significantly at [a]$P = 0.1$; [b]$P = 0.05$.

Table 9. Mean percentage of *"Cylindrocarpon"*-like fungi infection in the crown of the different cultivar combinations isolated from the nursery stone fruit trees.

Cultivar combination	Infection (%) [a]
Plum 1-1 [b]	58.33 [A]
Plum 3-1	44.17 [AB]
Plum 2-1	29.17 [BC]
Plum 1-2	27.50 [BC]
Nectarine 1-1	18.33 [CD]
Plum 1-3,2-3,3-3	10.00 [CD]
Plum 2-2	9.17 [CD]
Plum 3-2	1.67 [D]
Nectarine 1-2	0.00 [D]

[a] Means followed by the same letter do not differ significantly from each other at *P* = 0.05.

[b] First number in cultivar type refer to scion and second number to rootstock.

Table 10. Percentage latent infection of different fungal taxonomic groups in the nursery stone fruit trees.

Taxonomic group	Number of infected trees	Infected trees (%)
"Cylindrocarpon"-like fungi	255	23.61
Cadophora spp.	74	6.85
Botryosphaeriaceae	51	4.72
Amphisphaeriaceae	32	2.96
Coniochaetales	18	1.67
Basidiomycetes	16	1.48
Tympanidaceae	15	1.39
Paraphaeosphaeria spp.	12	1.11
Didymosphaeria spp.	11	1.02
Phaeoacremonium spp.	10	0.93
Valcaceae	9	0.80
Pleurostoma spp.	7	0.65
Diatrypaceae	4	0.37
Diaporthaceae	3	0.28
Paraphoma spp.	3	0.28
Neophaeomoniella spp.	1	0.09

Table 11. Mean percentage infection in the plant parts of the nursery stone fruit trees with *Cadophora* spp, Botryosphaeriaceae and Amphishaeriaceae.

Plant parts	Infection (%) [a]		
	Amphisphaeriaceae	Botryosphaeriaceae	*Cadophora* spp.
Wound	0.83 [B]	1.75 [B]	0.58 [B]
Bud union	0.63 [B]	2.92 [AB]	0.67 [B]
Crown	1.63 [B]	0.46 [B]	5.17 [A]

[a] Means followed by the same letter over the whole table do not differ significantly at P = 0.05.

Table 12. Infection with "*Cylindrocarpon*"-like fungi in the tissue culture rootstock (NP/TC) and hardwood rootstock (NP/HW) nursery stone fruit trees per plant part infected.

| Combination | Infected plant parts (%) | | |
| | "*Cylindrocarpon*"-like fungi | | |
	Wound	Bud union	Crown
NP/TC	0.0	0.0	5.0
NP/HW	0.0	5.0	25.0
P – value [a]	-	0.3112	0.0765

[a] Probability value calculated with Chi-square under the null hypothesis that infected plant parts are independent of cultivar combination.

Table 13. Fungal organisms found in the tissue culture rootstock (NP/TC) and hardwood rootstock cutting (NP/HW) nursery stone fruit trees.

Combination	Fungal organism	Plant parts infected (%)
NP/TC	*Coniochaeta hoffmannii*	1.7
	Diplodia seriata	3.3
	Neopestalotiopsis javaensis	1.7
	Paraphaeosphaeria sporulosa	1.7
	Thelonectria veuillotiana	1.7
NP/HW	*Coniochaeta* sp. 2	3.3
	Coprinellus micaceus	1.7
	Dactylonectria novozelandica	1.7
	Dactylonectria sp. 1	1.7
	Ilyonectria liriodendri	1.7
	Thelonectria truncata	5.0
	Thelonectria veuillotiana	1.7

Table 14. Mean lesion lengths and percentage re-isolation of fungal species in plum Orchard 1 (African Rose) 4 months after inoculation.

Fungal Species and Isolate STEU Number	Mean Lesion Length (mm)[a]	Log Transformed Mean		% Re-isolation Mean
		Mean[b]	Std Dev	
*Lasiodiplodia theobromae*_8849	229.94	5.44[A]	0.51	86
*Lasiodiplodia theobromae*_8850	227.65	5.43[A]	0.42	83
*Diaporthe ambigua*_8901	55.20	4.02[B]	0.29	60
*Diplodia seriata*_8948	49.40	3.91[BC]	0.22	61
*Diplodia seriata*_8846	48.90	3.90[BC]	0.23	60
*Biscogniauxia mediterranea*_8842	47.92	3.88[BCD]	0.36	32
*Diplodia seriata*_8949	47.44	3.87[BCD]	0.34	56
Biscogniauxia sp._8843	44.00	3.79[B-E]	0.33	23
*Cytospora leucostoma*_8882	40.76	3.72[C-F]	0.52	45
*Diaporthe foeniculina*_8904	39.54	3.69[C-G]	0.31	81
*Diplodia seriata*_8950	38.36	3.66[C-H]	0.24	66
*Truncatella angustata*_8834	37.59	3.64[C-I]	0.25	68
*Schizophyllum commune*_8841	36.47	3.61[D-J]	0.33	50
*Diplodia seriata*_8845	36.47	3.61[D-J]	0.37	68
*Cadophora luteo-olivacea*_8857	33.97	3.54[E-K]	0.43	54
Cytospora sp. 2_8886	33.97	3.54[E-L]	0.35	70
*Pleurostoma richardsiae*_8951	33.62	3.53[E-M]	0.34	84
*Phaeoacremonium iranianum*_8929	33.62	3.53[E-M]	0.42	76
*Cadophora luteo-olivacea*_8856	32.62	3.50[F-N]	0.53	78
Eutypella sp._8906	31.96	3.48[F-O]	0.37	5
*Phaeoacremonium iranianum*_8930	31.32	3.46[F-P]	0.31	74
*Phaeoacremonium parasiticum*_8934	30.69	3.44[F-Q]	0.34	60
*Didymosphaeria variabile*_8913	29.77	3.41[G-R]	0.26	65
*Cadophora gregata*_8855	29.77	3.41[G-S]	0.61	61
*Paraphaeosphaeria sporulosa*_8922	29.77	3.41[H-S]	0.29	46
*Pleurostoma richardsiae*_8937	29.77	3.41[H-S]	0.26	89
Cytospora sp. 1_8885	28.58	3.37[I-T]	0.33	59
*Didymosphaeria variabile*_8912	28.00	3.35[J-U]	0.28	55
*Paraphaeosphaeria sporulosa*_8923	27.16	3.32[K-V]	0.23	49
*Collophorina paarla*_8866	27.16	3.32[K-V]	0.26	54
*Diaporthe aspalathi*_8903	27.16	3.32[K-V]	0.27	51
Coniochaeta sp. 2_8876	26.61	3.30[K-V]	0.36	53
*Eutypa leptoplaca*_8905	26.61	3.30[K-V]	0.38	14
*Cytospora austromontana*_8880	26.34	3.29[K-V]	0.37	60
*Collophorina rubra*_8868	26.08	3.28[K-V]	0.20	63
*Valsa sordida*_8889	25.81	3.27[K-W]	0.25	51
*Phaeoacremonium parasiticum*_8933	25.81	3.27[K-W]	0.20	64
Coniochaeta sp. 2_8875	25.81	3.27[K-W]	0.31	59
*Coniochaeta hoffmannii*_8869	25.55	3.26[K-W]	0.43	55

*Cadophora novi-eboraci*_8860	25.55	3.26[K-W]	0.27	69
Cadophora sp. 2_8865	25.55	3.26[L-W]	0.40	76
*Paraphaeosphaeria neglecta*_8920	25.29	3.25[M-W]	0.38	73
*Didymosphaeria rubi-ulmifolii*_8911	25.29	3.25[M-W]	0.38	53
*Didymosphaeria rubi-ulmifolii*_8910	25.03	3.24[N-W]	0.24	54
*Paraphoma chrysanthemicola*_8924	25.03	3.24[N-W]	0.43	39
Cytospora sp. 2_8887	24.78	3.23[N-W]	0.31	60
Cadophora sp. 2_8864	24.78	3.23[N-W]	0.29	69
*Didymella pomorum*_8909	24.53	3.22[N-W]	0.43	39
*Diaporthe ambigua*_8902	24.53	3.22[N-W]	0.42	75
*Pleurostoma richardsiae*_8936	24.28	3.21[O-W]	0.30	75
*Cadophora gregata*_8854	24.03	3.20[O-W]	0.39	54
Cytospora sp. 1_8883	23.55	3.18[P-W]	0.14	69
*Cadophora malorum*_8859	23.55	3.18[P-W]	0.24	63
*Coniochaeta velutina*_8878	23.31	3.17[Q-W]	0.43	51
*Paraphaeosphaeria neglecta*_8921	23.31	3.17[Q-W]	0.20	41
*Truncatella angustata*_8835	23.07	3.16[Q-W]	0.38	31
*Coniochaeta hoffmannii*_8870	22.84	3.15[R-W]	0.44	19
*Cytospora leucostoma*_8881	22.60	3.14[R-W]	0.25	54
*Dothiorella viticola*_8848	22.37	3.13[S-W]	0.40	25
*Coniochaeta velutina*_8879	21.70	3.10[T-W]	0.28	69
*Paraphoma chrysanthemicola*_8925	21.48	3.09[T-W]	0.21	26
*Cadophora malorum*_8858	21.04	3.07[UVW]	0.33	56
Coniochaeta sp. 1_8873	21.04	3.07[UVW]	0.48	48
*Dothiorella moneti*_8847	20.83	3.06[VW]	0.41	41
*Valsa sordida*_8888	20.83	3.06[VW]	0.28	73
*Didymella pomorum*_8908	19.59	3.00[W]	0.31	41
Control (PDA+s)	14.38	2.70[X]	0.18	0

Least Significant Difference (LSD) of Logarithmic transformed lesion lengths = 0.2838.
[a] Back transformed mean lesion length.
[b] Means followed by the same letter do not differ significantly at *P* = 0.05. Means represent the average of 10 replicates per fungal isolate.

Table 15. Mean lesion lengths and percentage re-isolation of fungal species in plum Orchard 2 (Sunkiss) 4 months after inoculation.

Fungal Species and Isolate STEU Number	Mean Lesion Length (mm)[a]	Log Transformed Mean		% Re-isolation Mean
		Mean[b]	Std Dev	
*Lasiodiplodia theobromae*_8850	191.98	5.26[A]	0.56	66
*Lasiodiplodia theobromae*_8849	136.50	4.92[B]	0.51	68
*Biscogniauxia mediterranea*_8842	100.99	4.62[B]	0.49	78
*Diplodia seriata*_8949	57.47	4.06[C]	0.51	80
Biscogniauxia sp._8843	48.41	3.89[CD]	0.35	66
*Diplodia seriata*_8948	46.49	3.85[CDE]	0.44	59
*Collophorina paarla*_8866	44.65	3.81[C-F]	0.25	90
*Diplodia seriata*_8950	43.76	3.79[C-G]	0.44	64
Eutypella sp._8906	41.18	3.73[D-H]	0.43	21
Cadophora sp. 2_8864	41.18	3.73[D-I]	0.37	90
Cadophora sp. 2_8865	40.76	3.72[D-J]	0.27	93
*Phaeoacremonium iranianum*_8929	39.15	3.68[D-K]	0.35	78
*Phaeoacremonium parasiticum*_8934	38.36	3.66[D-L]	0.40	73
*Cadophora novi-eboraci*_8860	37.59	3.64[D-M]	0.29	84
*Cadophora luteo-olivacea*_8856	37.21	3.63[D-M]	0.39	79
*Phaeoacremonium parasiticum*_8933	36.84	3.62[D-M]	0.42	93
*Cadophora luteo-olivacea*_8857	36.84	3.62[D-M]	0.27	81
*Coniochaeta hoffmannii*_8870	36.84	3.62[D-N]	0.43	64
*Schizophyllum commune*_8841	36.10	3.60[D-N]	0.33	84
*Cytospora leucostoma*_8882	35.02	3.57[D-O]	0.41	38
*Pleurostoma richardsiae*_8936	34.66	3.56[D-O]	0.36	100
*Diaporthe aspalathi*_8903	34.66	3.56[D-O]	0.23	53
*Diplodia seriata*_8845	34.66	3.56[D-O]	0.48	68
Coniochaeta sp. 1_8873	34.66	3.56[E-P]	0.43	58
*Truncatella angustata*_8834	34.31	3.55[E-P]	0.23	56
*Coniochaeta hoffmannii*_8869	34.31	3.55[E-P]	0.43	81
*Cadophora gregata*_8855	33.97	3.54[E-Q]	0.43	78
*Diplodia seriata*_8846	33.97	3.54[E-Q]	0.40	78
*Coniochaeta velutina*_8879	33.62	3.53[E-Q]	0.43	76
*Didymosphaeria variabile*_8912	33.62	3.53[E-Q]	0.27	74
*Paraphaeosphaeria sporulosa*_8923	33.62	3.53[E-Q]	0.30	66
*Cadophora malorum*_8858	33.28	3.52[F-Q]	0.26	76
*Pleurostoma richardsiae*_8951	33.28	3.52[F-R]	0.32	91
*Diaporthe ambigua*_8901	32.62	3.50[F-R]	0.25	75
Cytospora sp. 1_8885	32.62	3.50[F-R]	0.34	64

*Cytospora austromontana*_8880	32.62	3.50[F-R]	0.36	61
*Cadophora malorum*_8859	32.62	3.50[F-R]	0.44	79
Cytospora sp. 1_8883	32.29	3.49[F-R]	0.36	70
*Paraphaeosphaeria sporulosa*_8922	31.96	3.48[G-R]	0.32	61
*Phaeoacremonium iranianum*_8930	31.96	3.48[G-R]	0.31	94
Cytospora sp. 2_8887	31.00	3.45[H-S]	0.34	84
*Valsa sordida*_8888	31.00	3.45[H-S]	0.39	44
*Collophorina rubra*_8868	31.00	3.45[H-S]	0.38	75
*Dothiorella viticola*_8848	31.00	3.45[H-S]	0.54	24
*Pleurostoma richardsiae*_8937	30.69	3.44[H-S]	0.28	89
*Eutypa leptoplaca*_8905	30.38	3.43[H-S]	0.27	36
Coniochaeta sp. 2_8876	29.77	3.41[H-S]	0.35	71
*Diaporthe ambigua*_8902	29.46	3.40[I-S]	0.19	75
*Didymosphaeria rubi-ulmifolii*_8911	29.46	3.40[J-S]	0.35	58
*Valsa sordida*_8889	29.46	3.40[J-S]	0.29	56
*Cadophora gregata*_8854	29.46	3.40[J-S]	0.51	81
Coniochaeta sp. 2_8875	28.87	3.38[K-S]	0.19	81
Cytospora sp. 2_8886	28.87	3.38[K-S]	0.56	65
*Didymosphaeria rubi-ulmifolii*_8910	28.58	3.37[K-S]	0.35	71
*Paraphaeosphaeria neglecta*_8921	28.29	3.36[K-S]	0.32	64
*Truncatella angustata*_8835	28.00	3.35[L-S]	0.28	46
*Cytospora leucostoma*_8881	26.89	3.31[M-S]	0.41	49
*Didymella pomorum*_8908	26.34	3.29[N-S]	0.27	68
*Didymella pomorum*_8909	26.34	3.29[N-S]	0.54	56
*Paraphaeosphaeria neglecta*_8920	25.81	3.27[O-S]	0.34	63
*Paraphoma chrysanthemicola*_8924	25.55	3.26[O-S]	0.32	43
*Diaporthe foeniculina*_8904	24.78	3.23[P-S]	0.29	69
*Coniochaeta velutina*_8878	24.53	3.22[QRS]	0.55	90
*Paraphoma chrysanthemicola*_8925	24.53	3.22[QRS]	0.17	63
*Didymosphaeria variabile*_8913	23.79	3.19[RS]	0.34	79
*Dothiorella moneti*_8847	22.60	3.14[S]	0.41	36
Control (PDA+s)	10.86	2.43[T]	0.19	0

Least Significant Difference (LSD) of Logarithmic transformed lesion lengths = 0.3259
[a] Back transformed mean lesion length.
[b] Means followed by the same letter do not differ significantly at *P* = 0.05. Means represent the average of 10 replicates per fungal isolate.
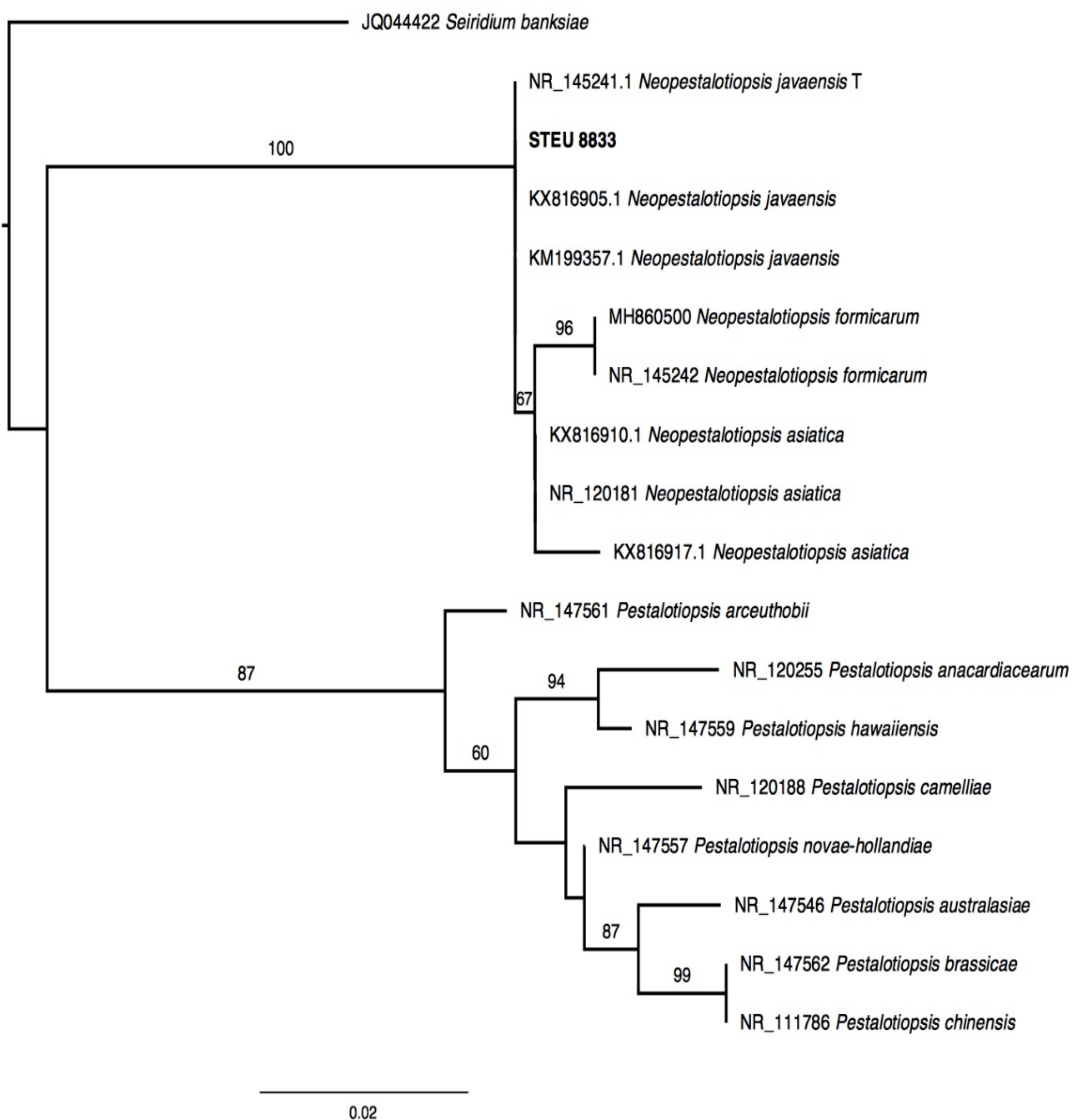
Figure 1. Maximum likelihood phylogenetic tree of *Neopestalotiopsis* species based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Seiridium banksiae* was used as outgroup and the isolate obtained in this study is indicated in bold.
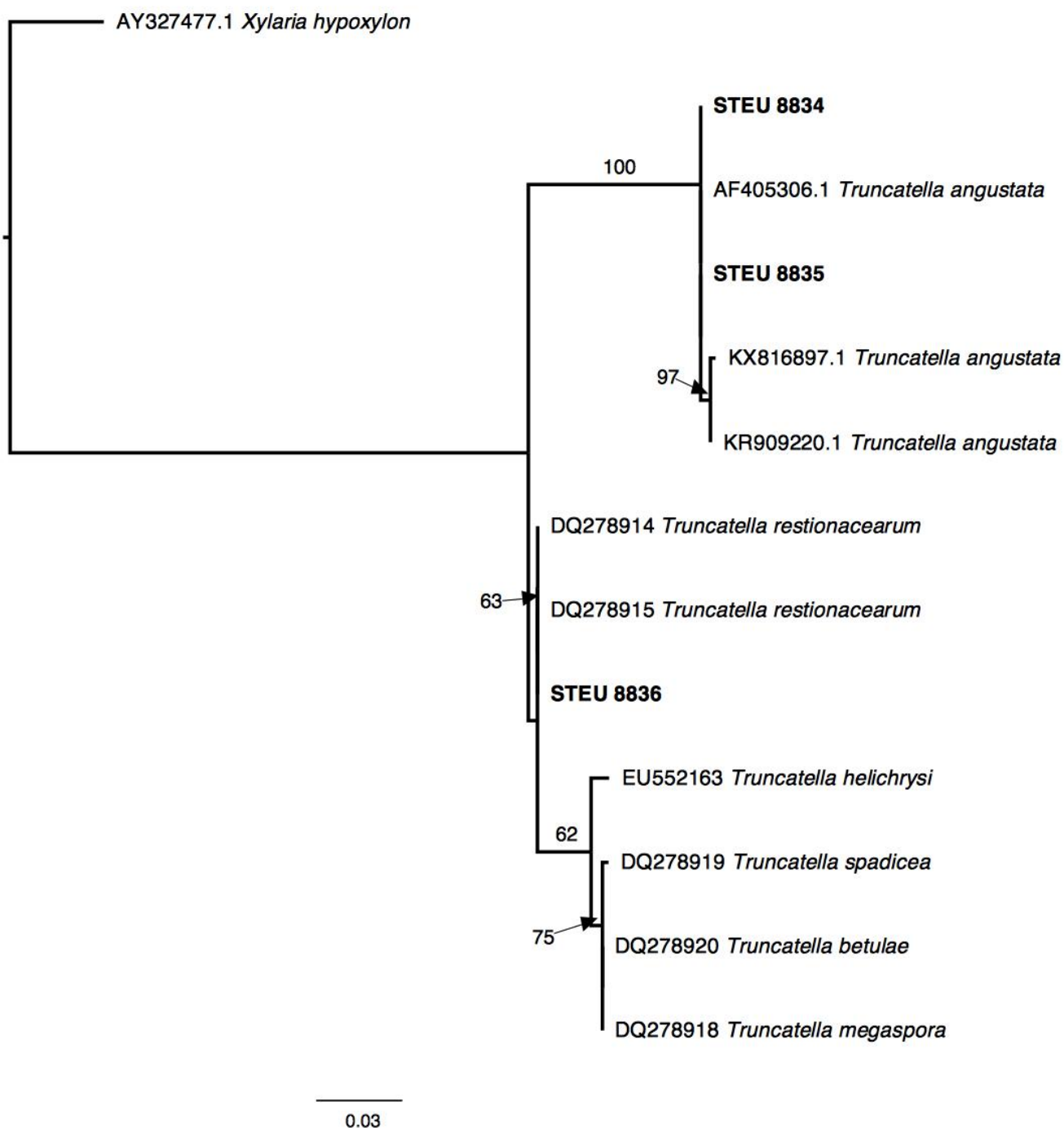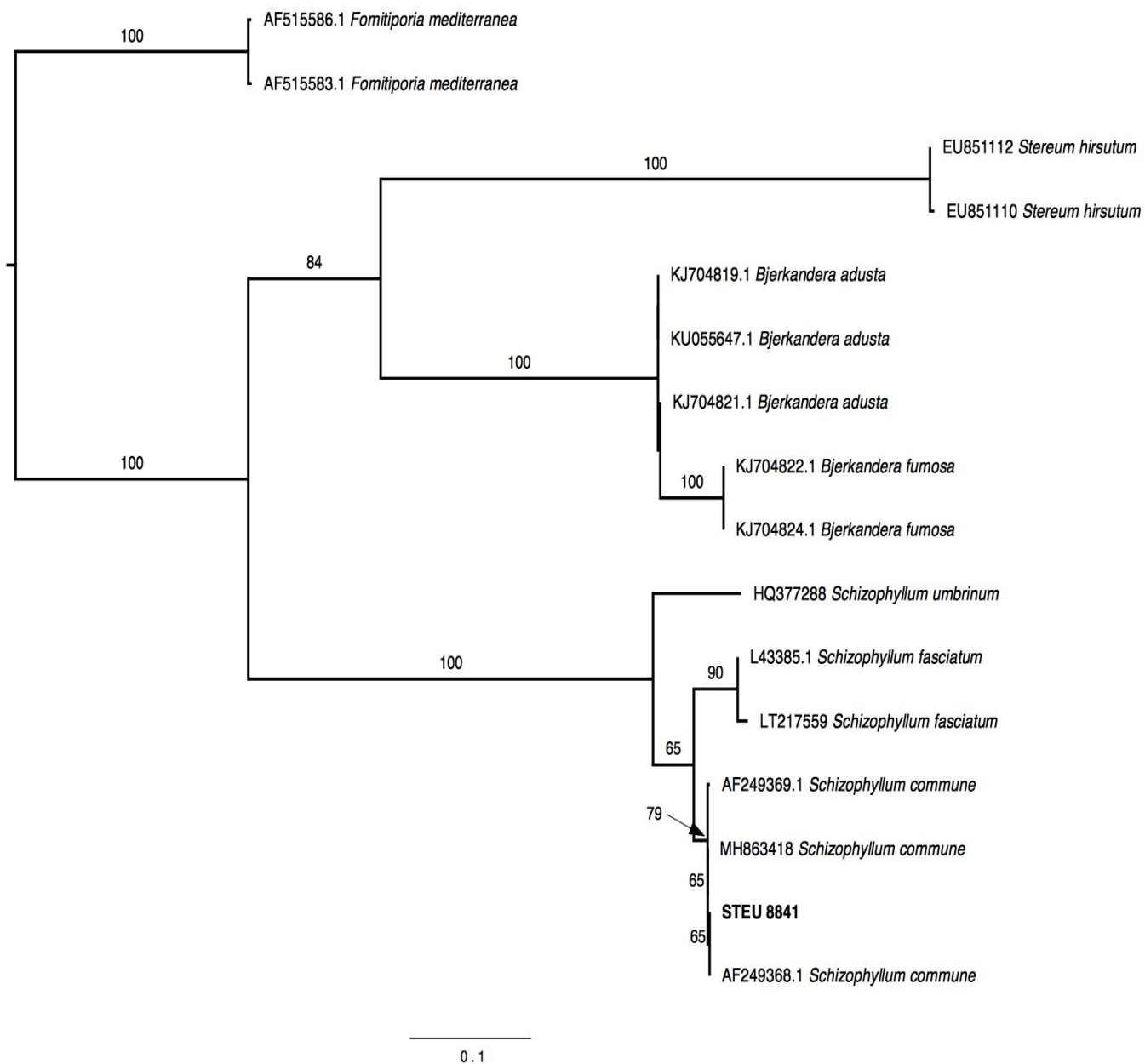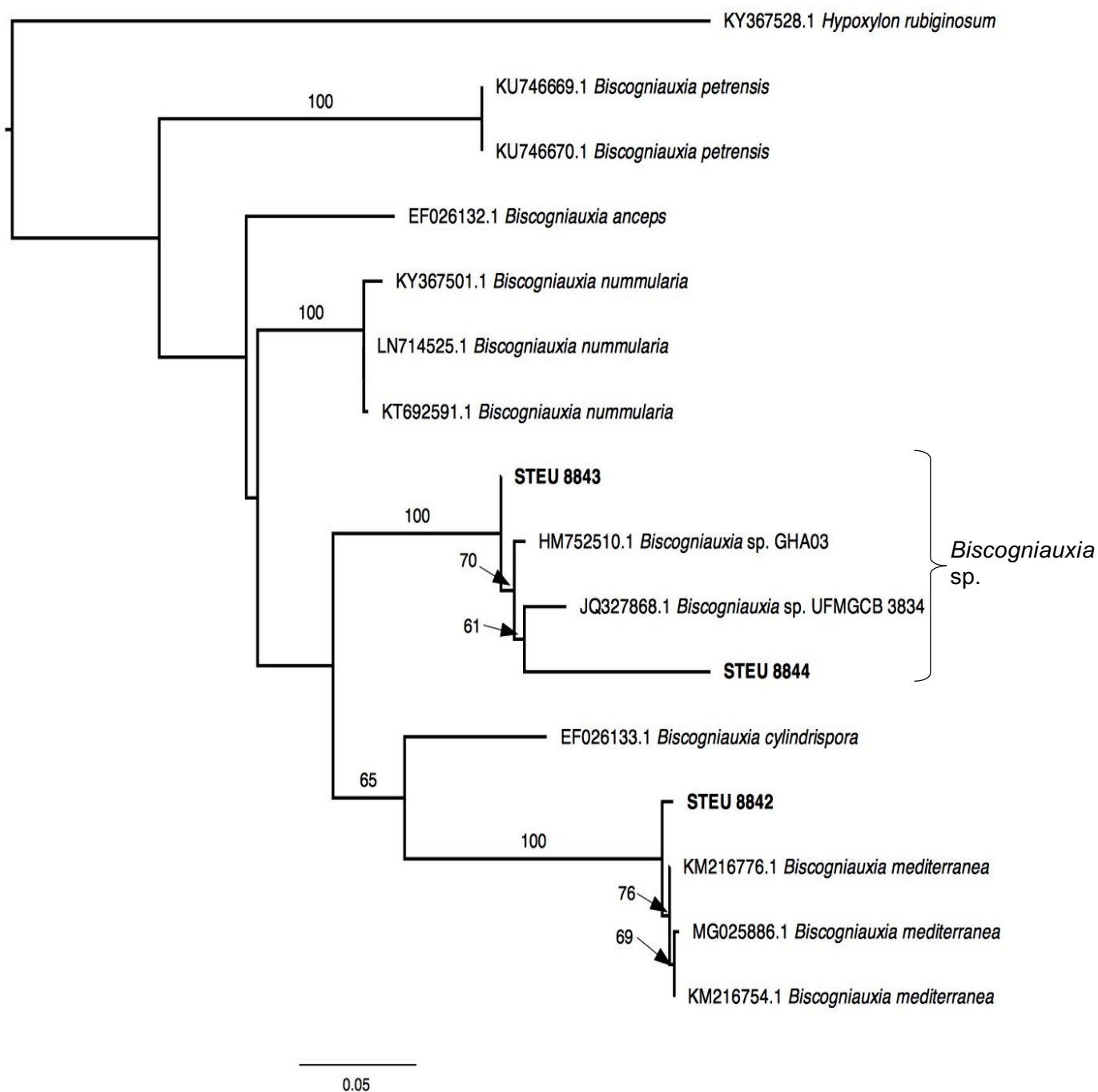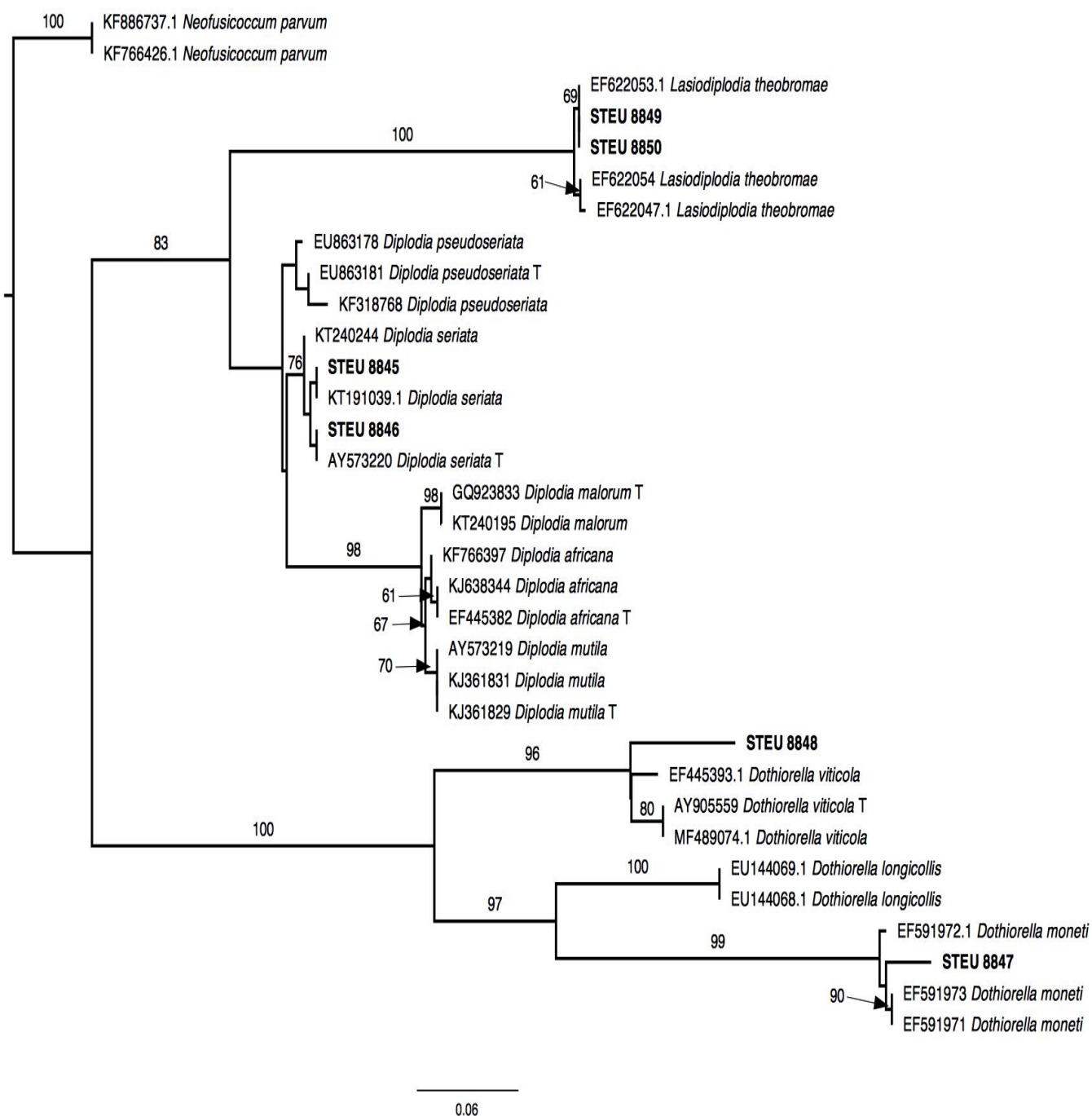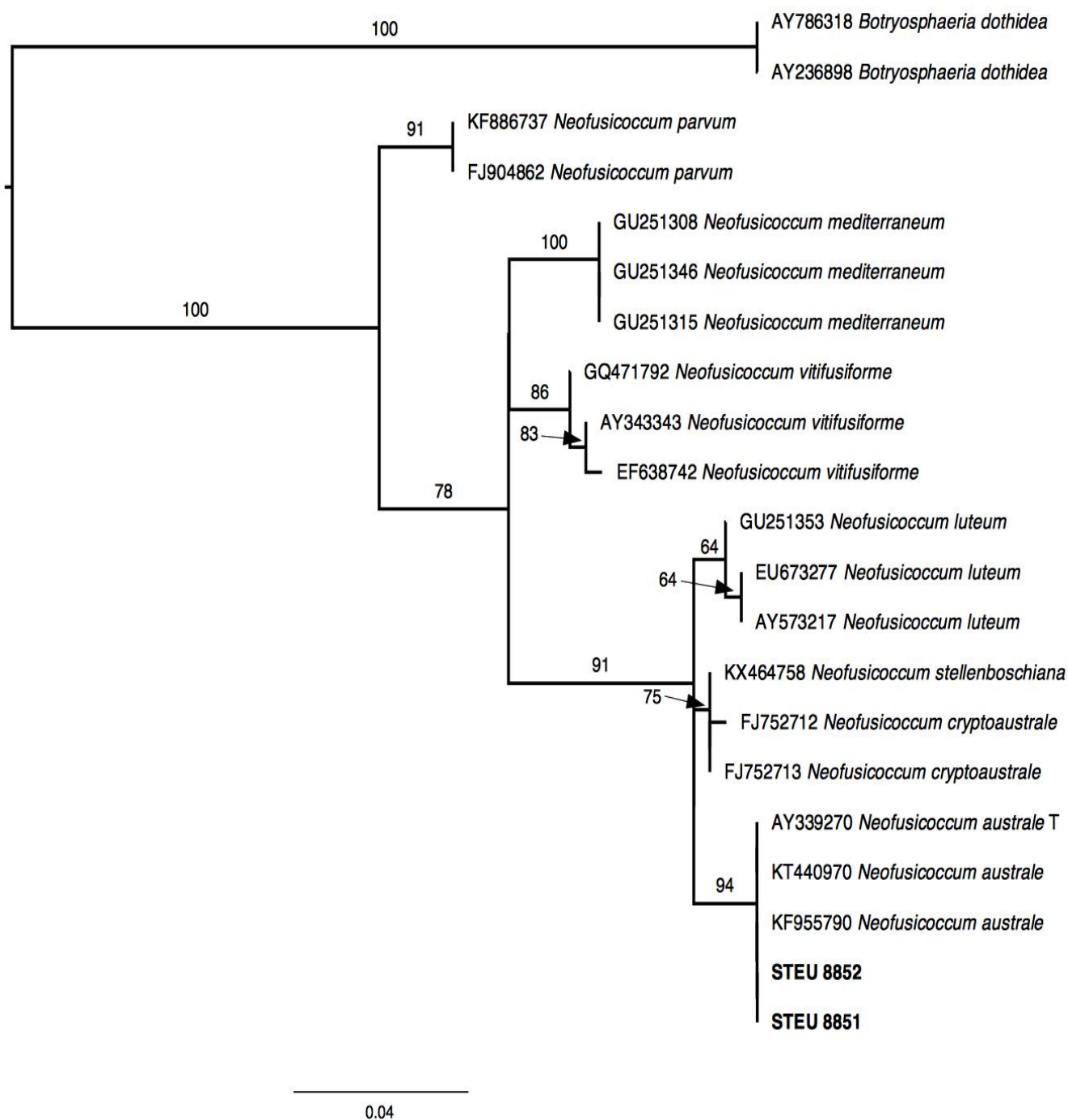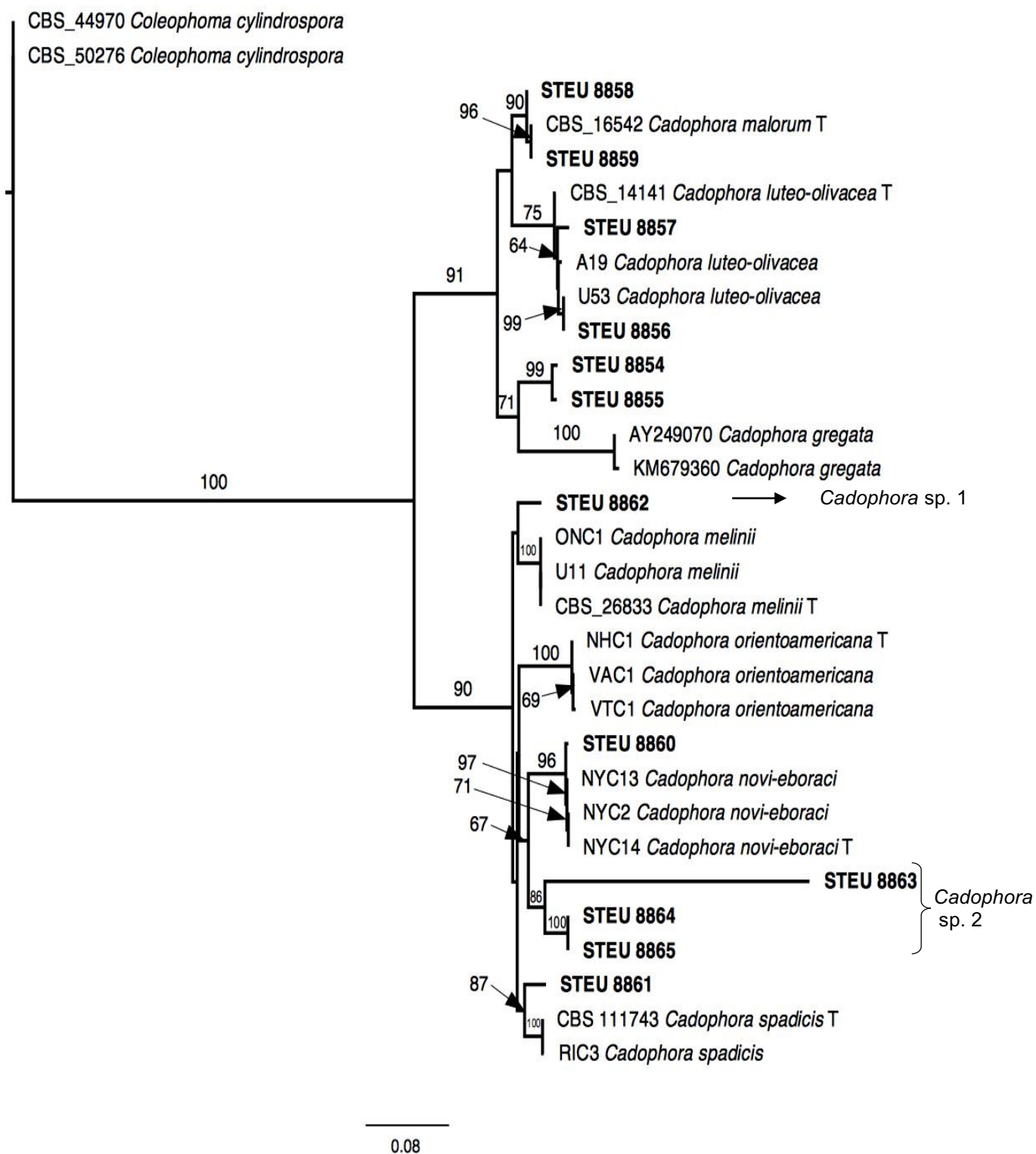
Figure 2. Maximum likelihood phylogenetic tree of *Truncatella* species based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Xylaria hypoxylon* was used as outgroup and the isolates obtained in this study are indicated in bold.
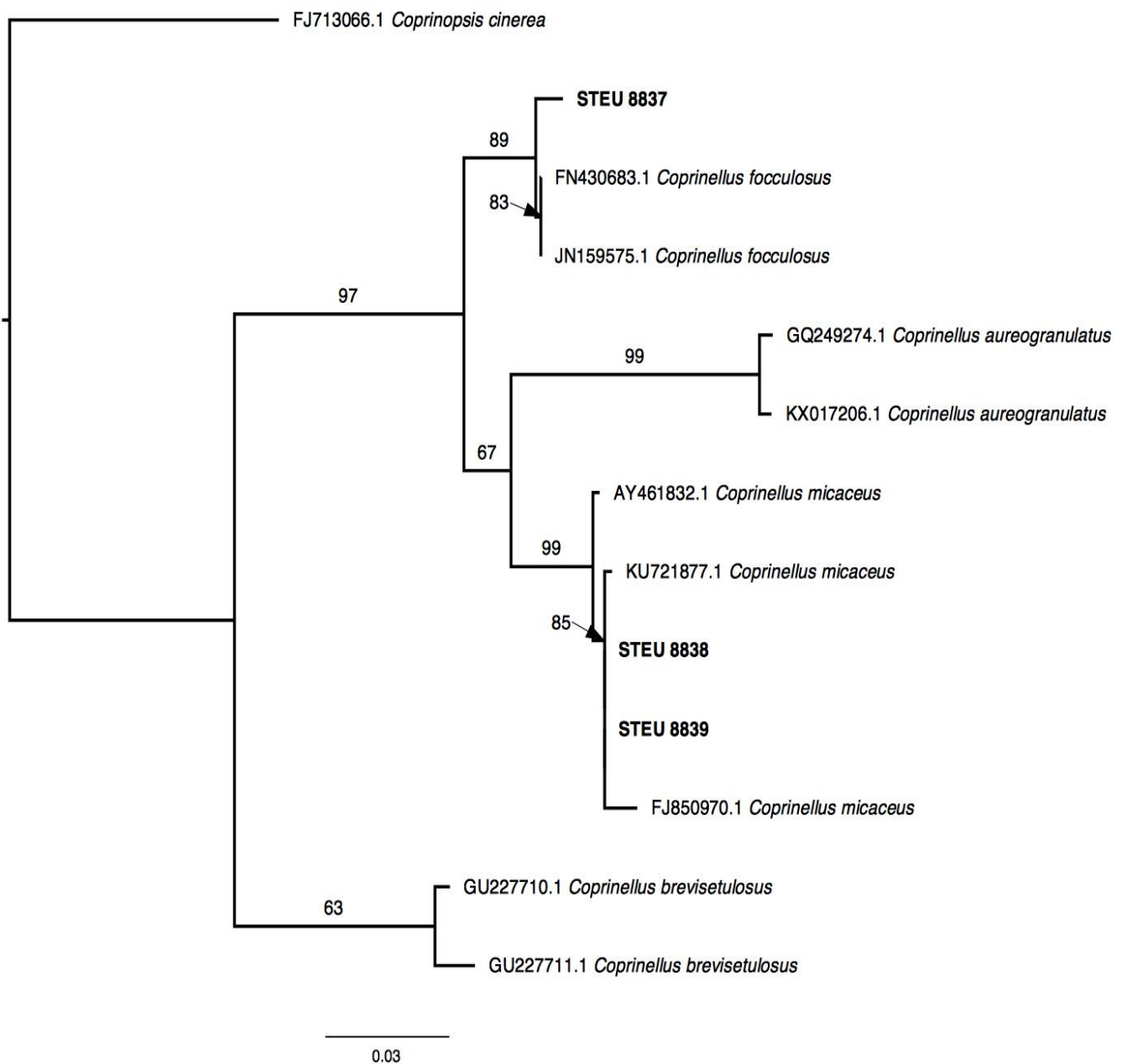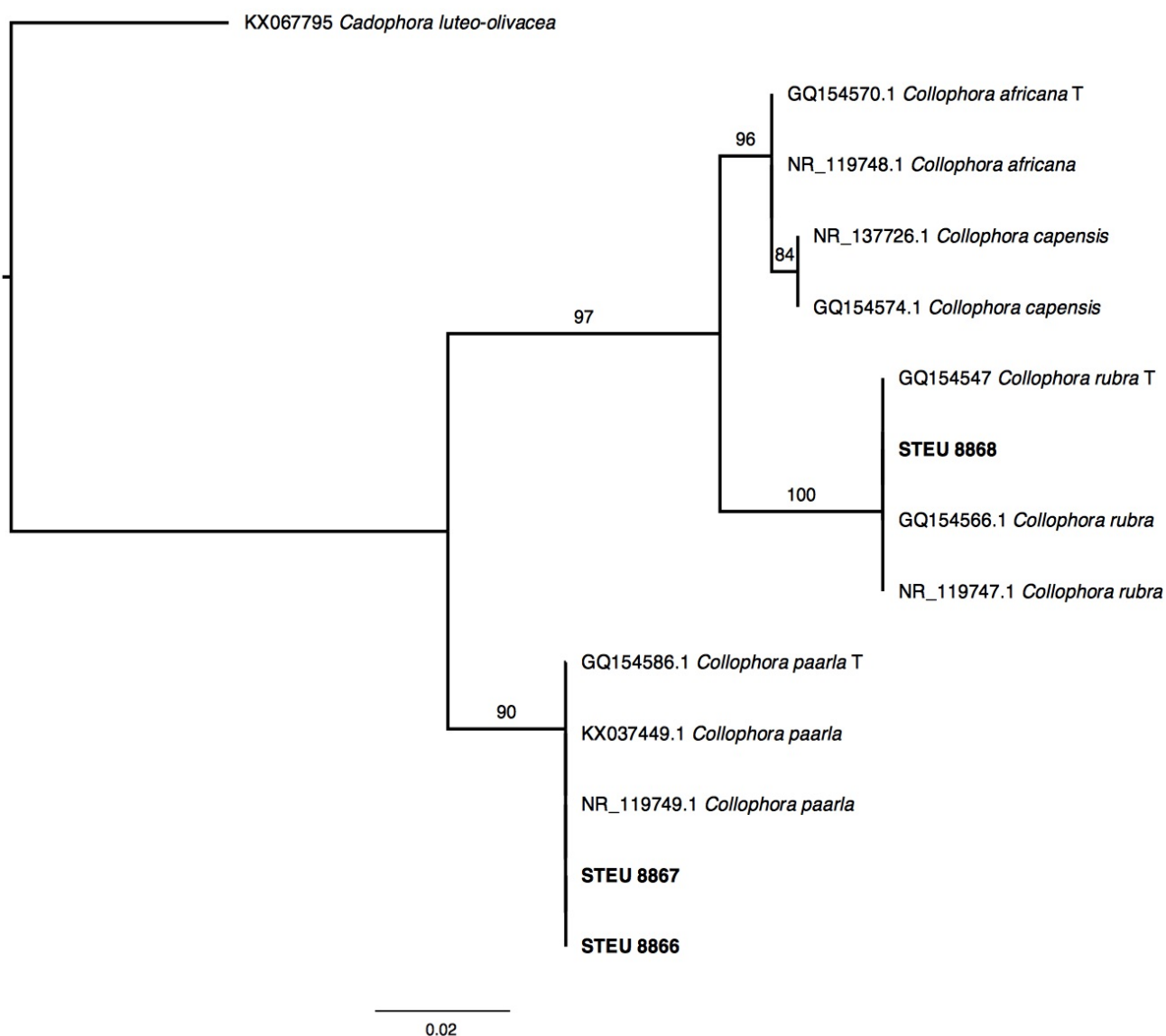
Figure 3. Maximum likelihood phylogenetic tree of Basidiomycetes, specifically of *Coprinellus* species, based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Coprinopsis cinerea* was used as outgroup and the isolates obtained in this study are indicated in bold.
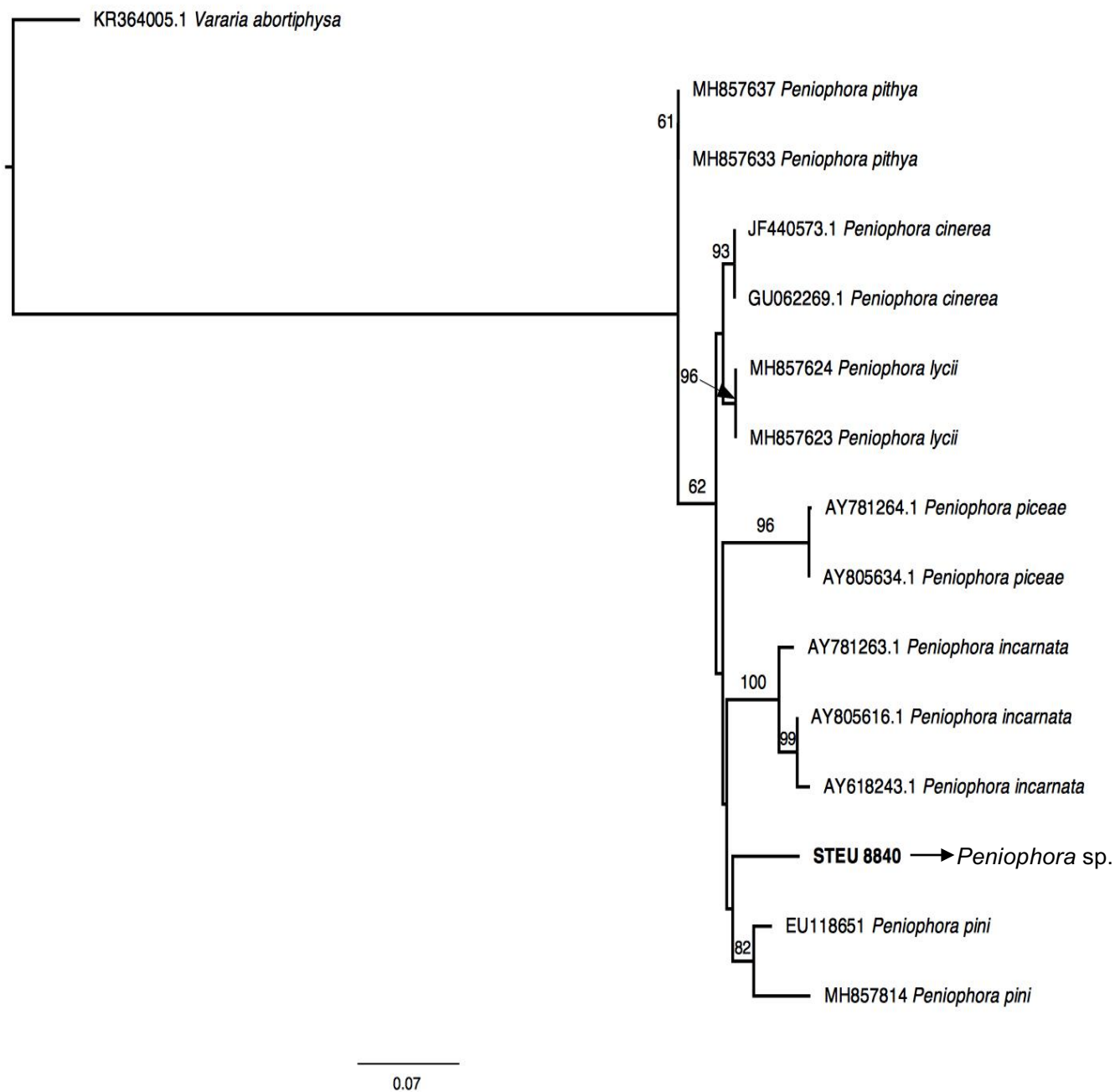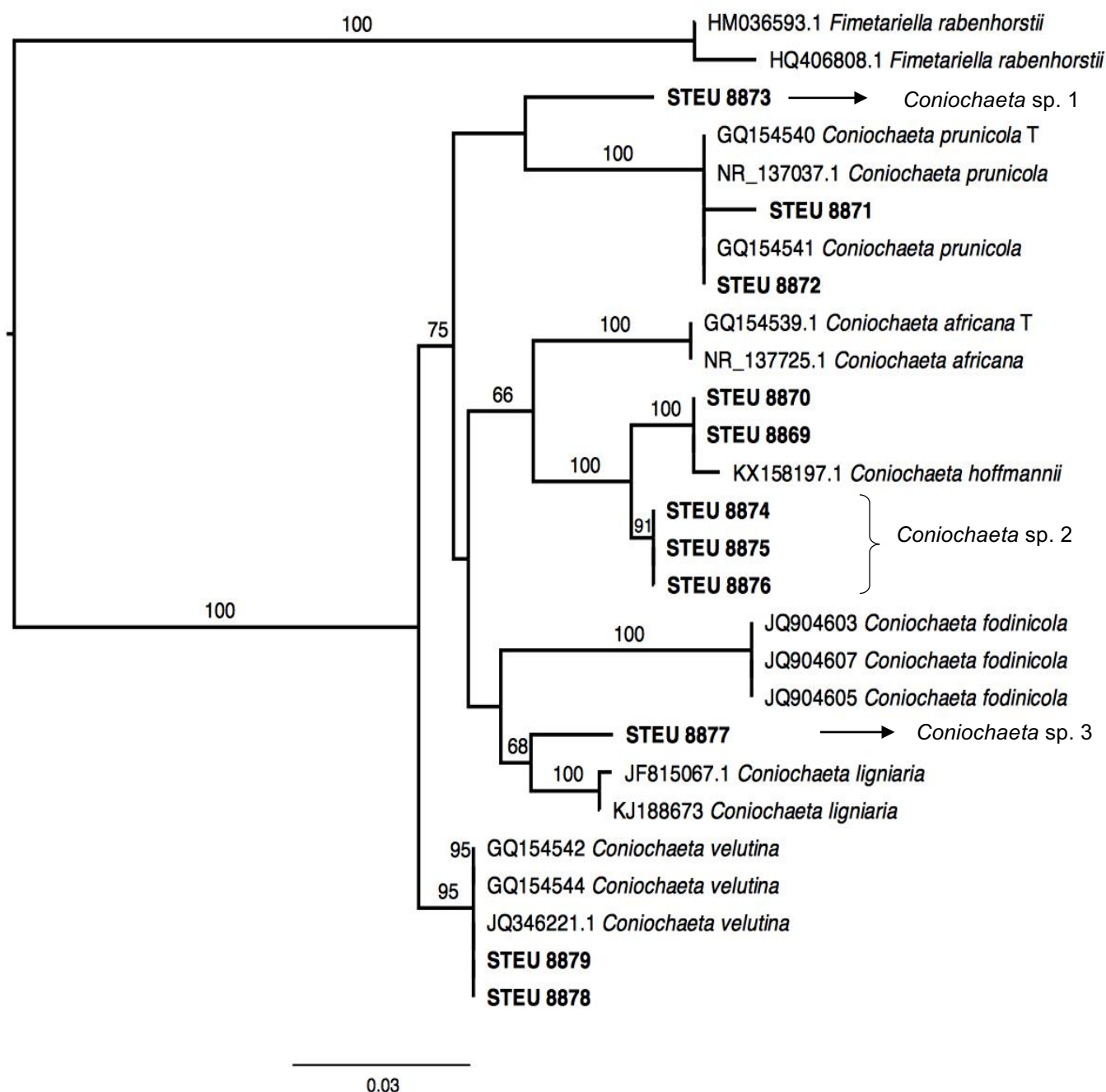
Figure 4. Maximum likelihood phylogenetic tree of *Peniophora* species, based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Vararia abortiphysa* was used as outgroup and the isolate obtained in this study is indicated in bold.

Figure 5. Maximum likelihood phylogenetic tree of Basidiomycetes (*Schizophyllum*, *Bjerkandera* and *Stereum*) based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Fomitoporia mediterranea* was used as outgroup and the isolate obtained in this study is indicated in bold.

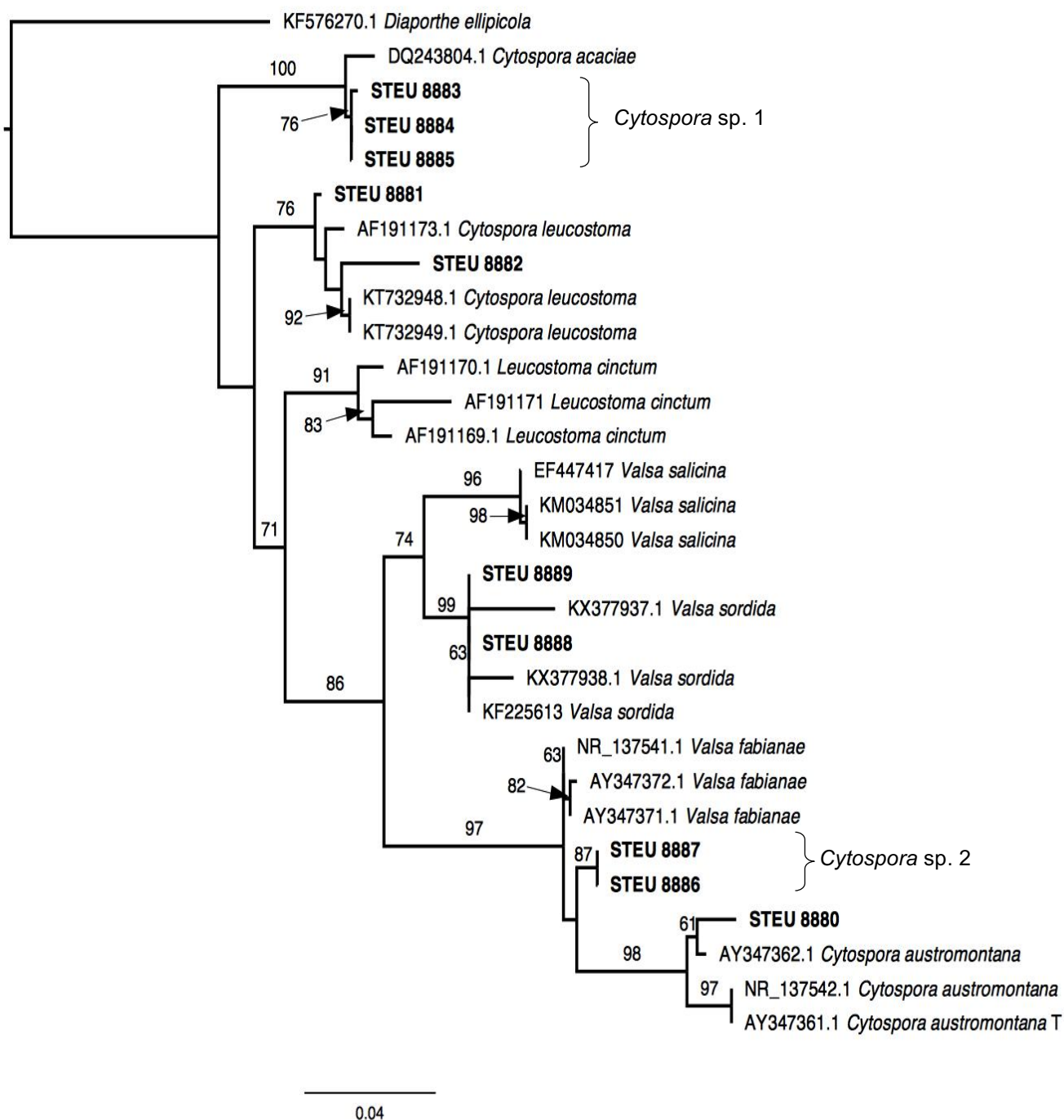**Figure 6.** Maximum likelihood phylogenetic tree of *Biscogniauxia* species based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Hypoxylon rubiginosum* was used as outgroup and the isolates obtained in this study are indicated in bold.
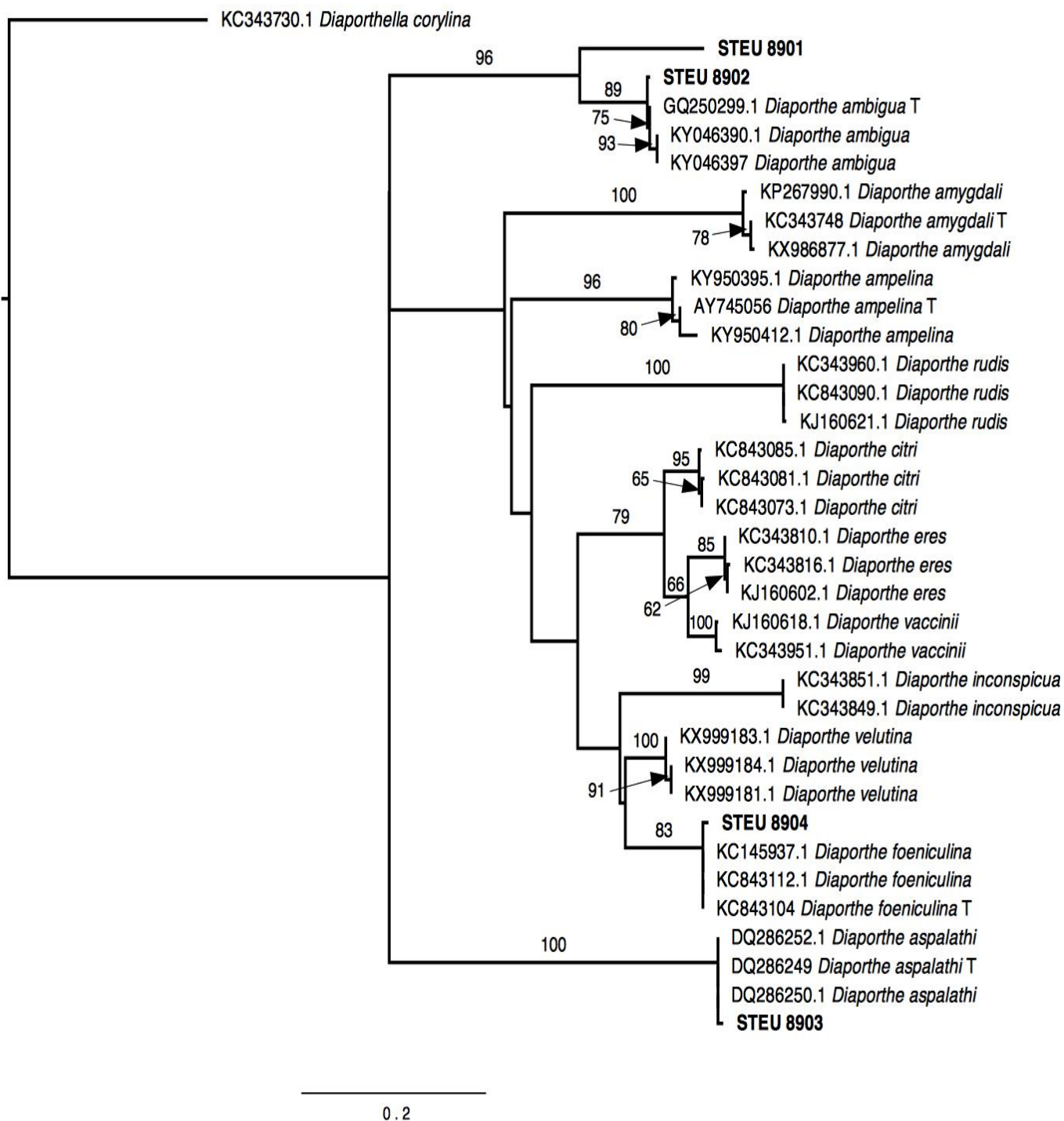
Figure 7. Maximum likelihood phylogenetic tree of Botryosphaeriaceae based on elongation factor 1-alpha sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Neofusicoccum parvum* was used as outgroup and the isolates obtained in this study are indicated in bold.

Figure 8. Maximum likelihood phylogenetic tree of *Neofusicoccum* species based on elongation factor 1-alpha sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Botryosphaeria dothidea* was used as outgroup and the isolates obtained in this study are indicated in bold.
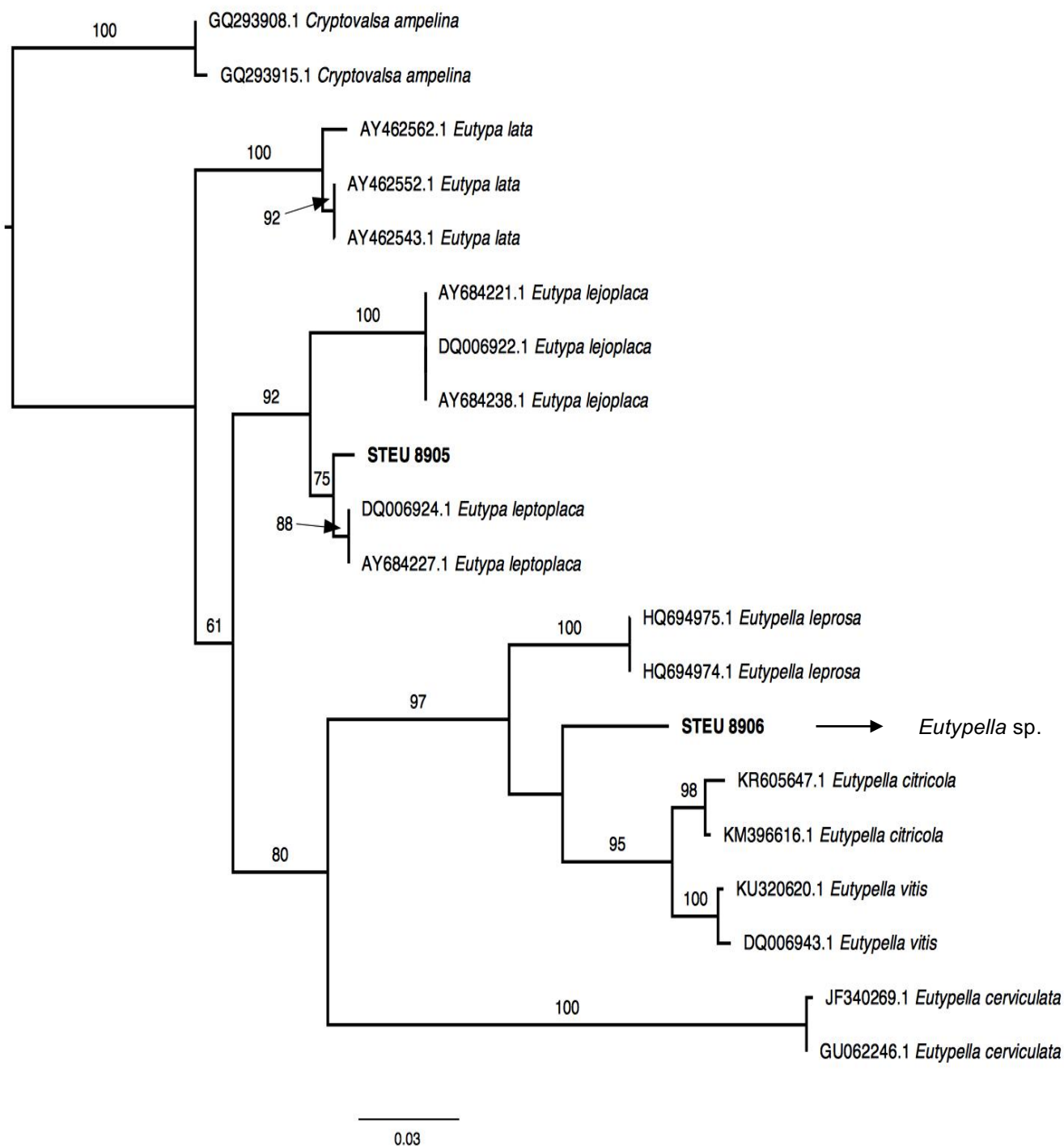
Figure 9. Maximum likelihood phylogenetic tree of *Cadophora* species based on ITS and elongation factor 1-alpha sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Coleophoma cylindrospora* was used as outgroup and the isolates obtained in this study are indicated in bold.
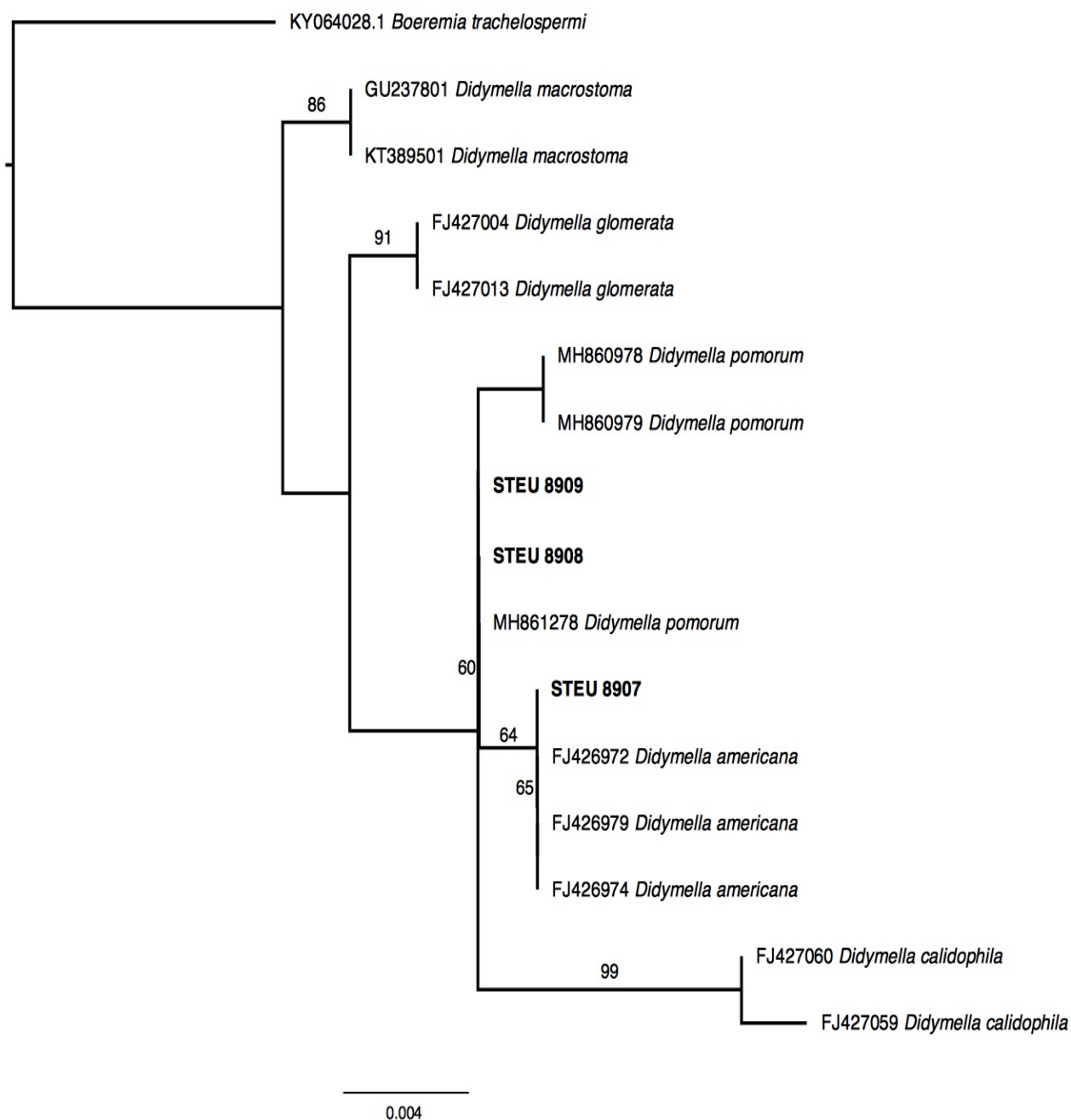
Figure 10. Maximum likelihood phylogenetic tree of *Collophorina* species (synonym *Collophora* species) based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Cadophora luteo-olivacea* was used as outgroup and the isolates obtained in this study are indicated in bold.

Figure 11. Maximum likelihood phylogenetic tree of *Coniochaeta* species based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Fimetariella rabenhorstii* was used as outgroup and the isolates obtained in this study are indicated in bold.
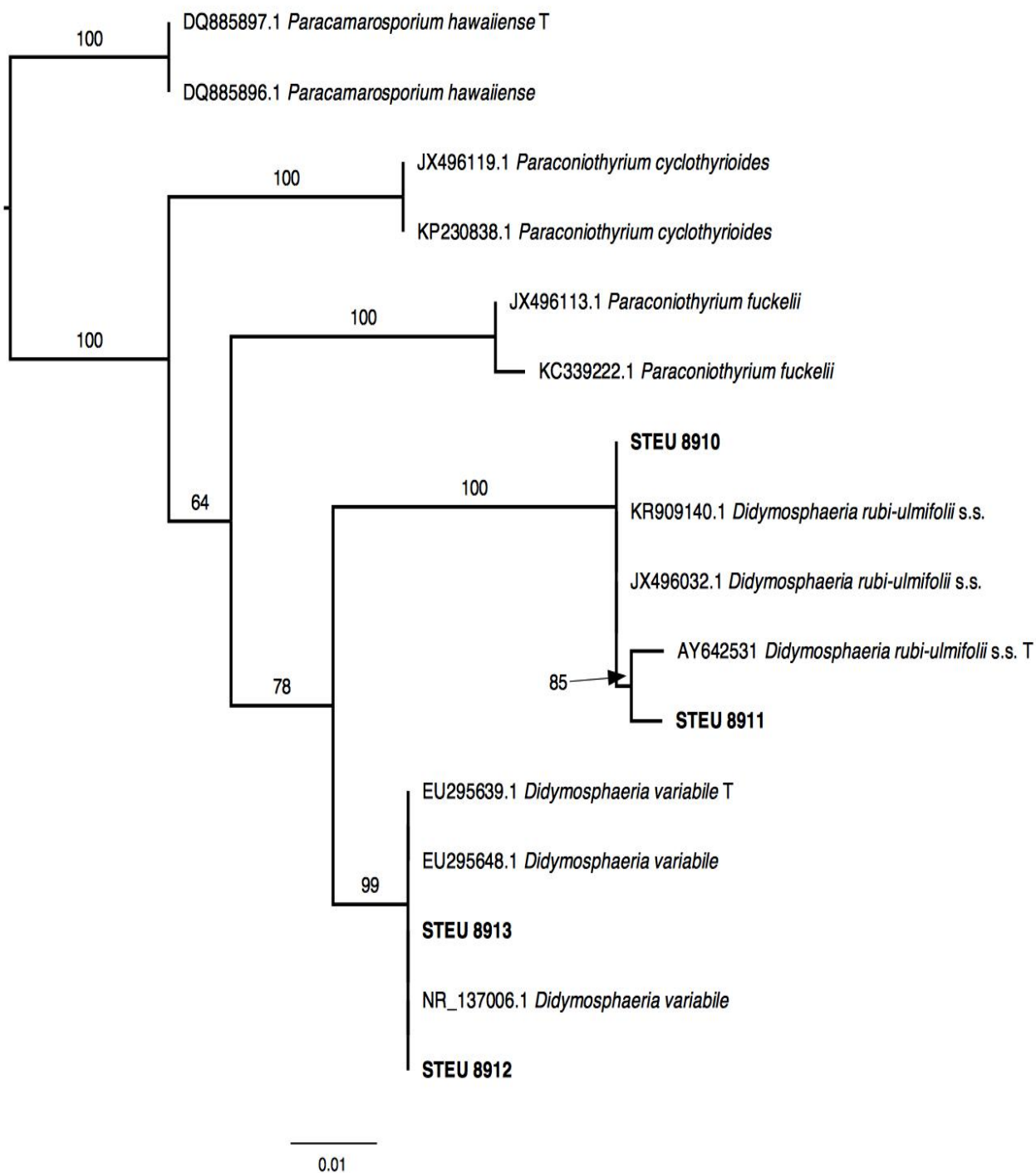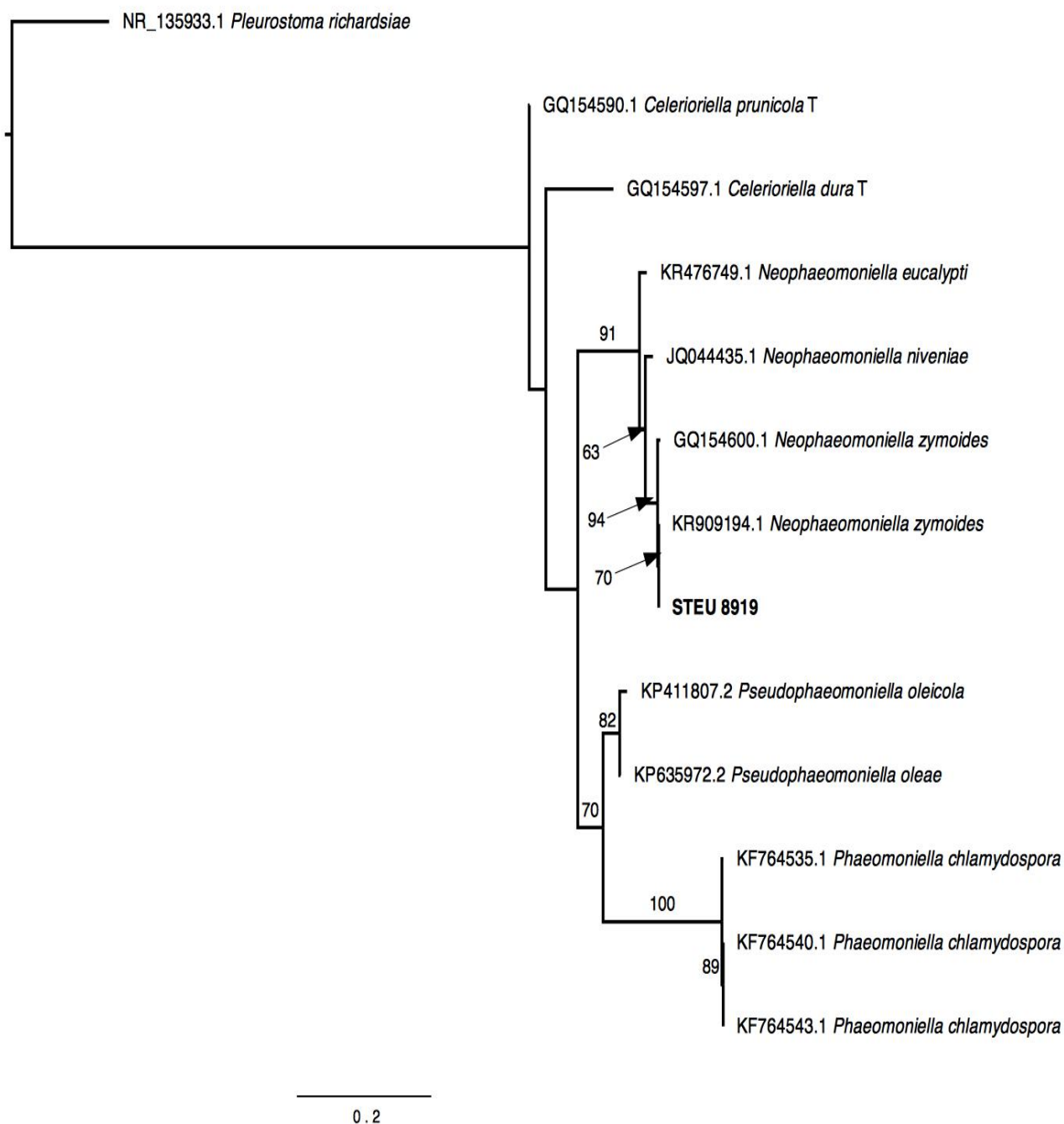
Figure 12. Maximum likelihood phylogenetic tree of Valsaceae species based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Diaporthe ellipicola* was used as outgroup and the isolates obtained in this study are indicated in bold.

Figure 13. Maximum likelihood phylogenetic tree of Diaporthaceae species based on elongation factor 1-alpha sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Diaporthella corylina* was used as outgroup and the isolates obtained in this study are indicated in bold.
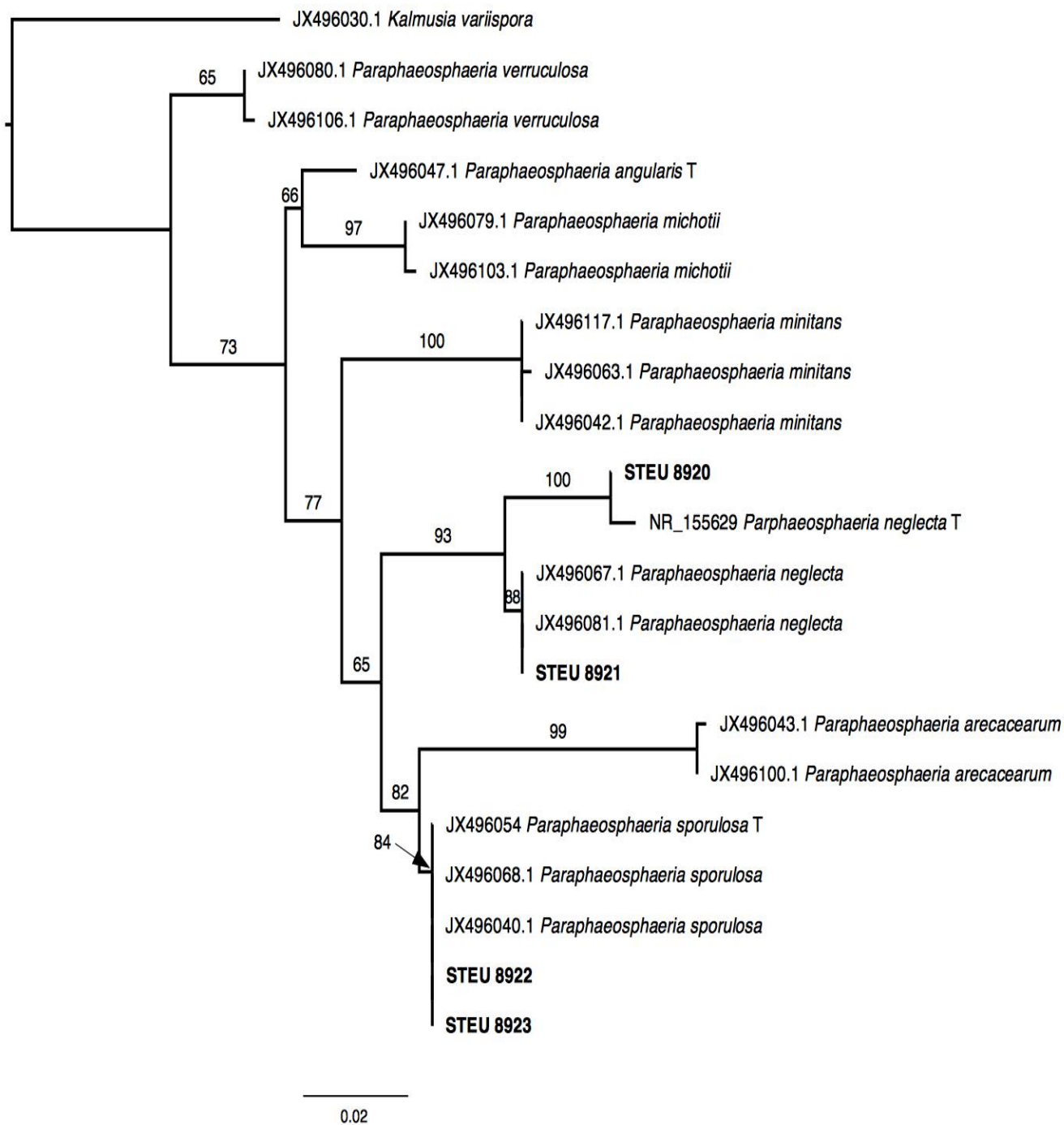
Figure 14. Maximum likelihood phylogenetic tree of Diatrypaceae based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Cryptovalsa ampelina* was used as outgroup and the isolates obtained in this study are indicated in bold.
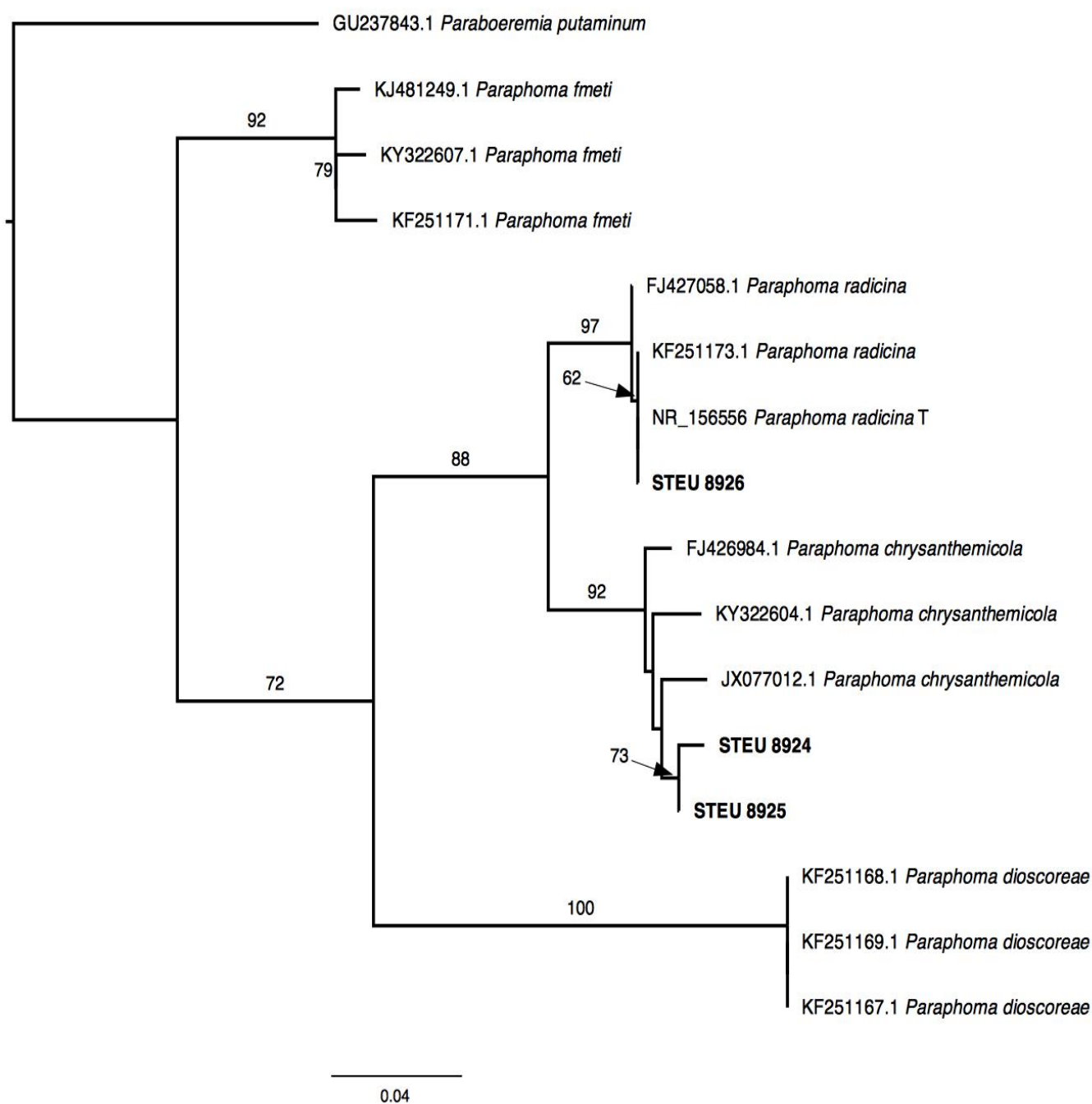
Figure 15. Maximum likelihood phylogenetic tree of *Didymella* species based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Boeremia trachelospermi* was used as outgroup and the isolates obtained in this study are indicated in bold.

Figure 16. Maximum likelihood phylogenetic tree of *Didymosphaeria* and *Paraconiothyrium* species based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Paracamarosporium hawaiiense* was used as outgroup and the isolates obtained in this study are indicated in bold.
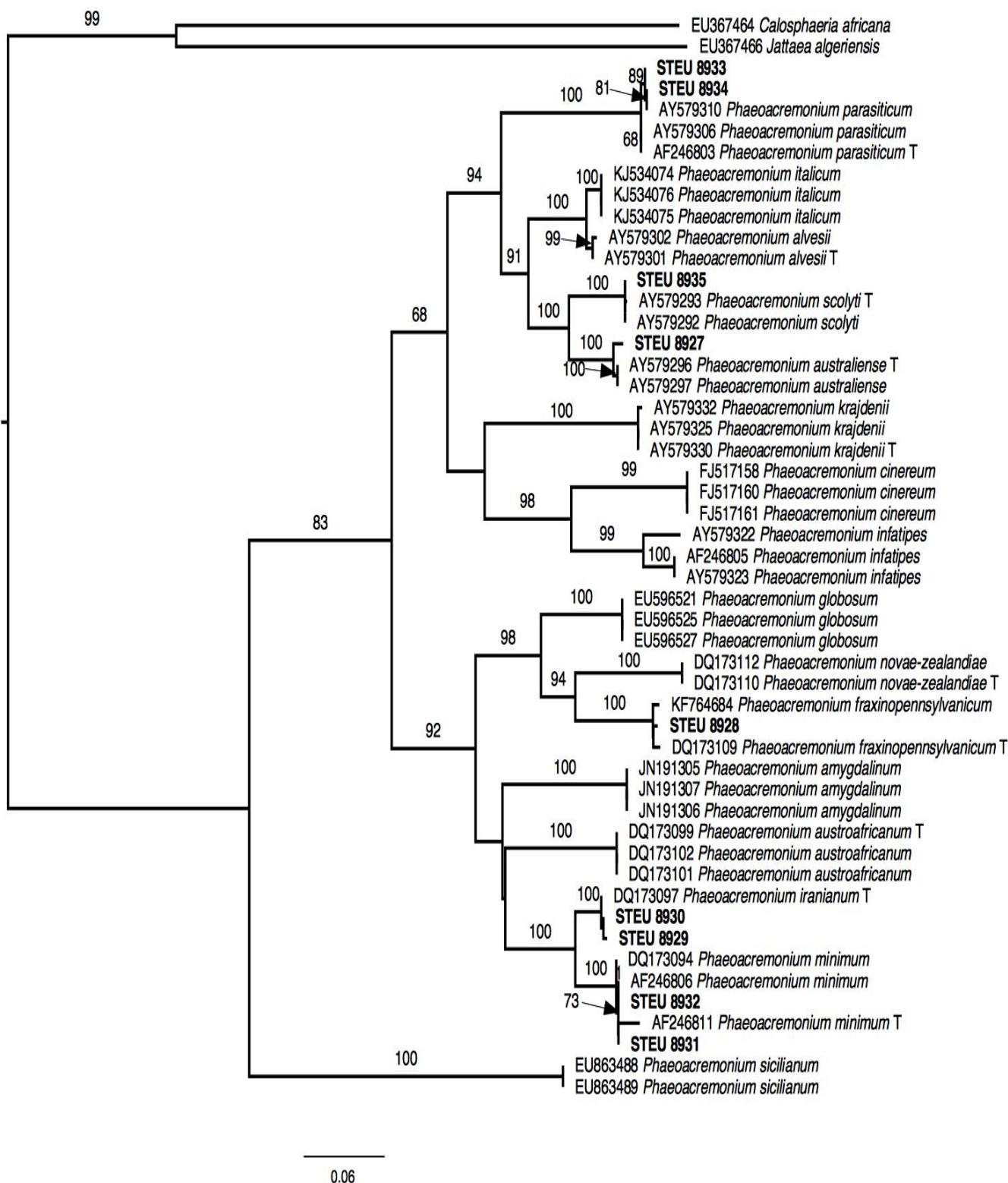
Figure 17. Maximum likelihood phylogenetic tree of *Neophaeomoniella* species based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Pleurostoma richardsiae* was used as outgroup and the isolate obtained in this study is indicated in bold.

Figure 18. Maximum likelihood phylogenetic tree of *Paraphaeosphaeria* species based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Kalmusia variispora* was used as outgroup and the isolates obtained in this study are indicated in bold.
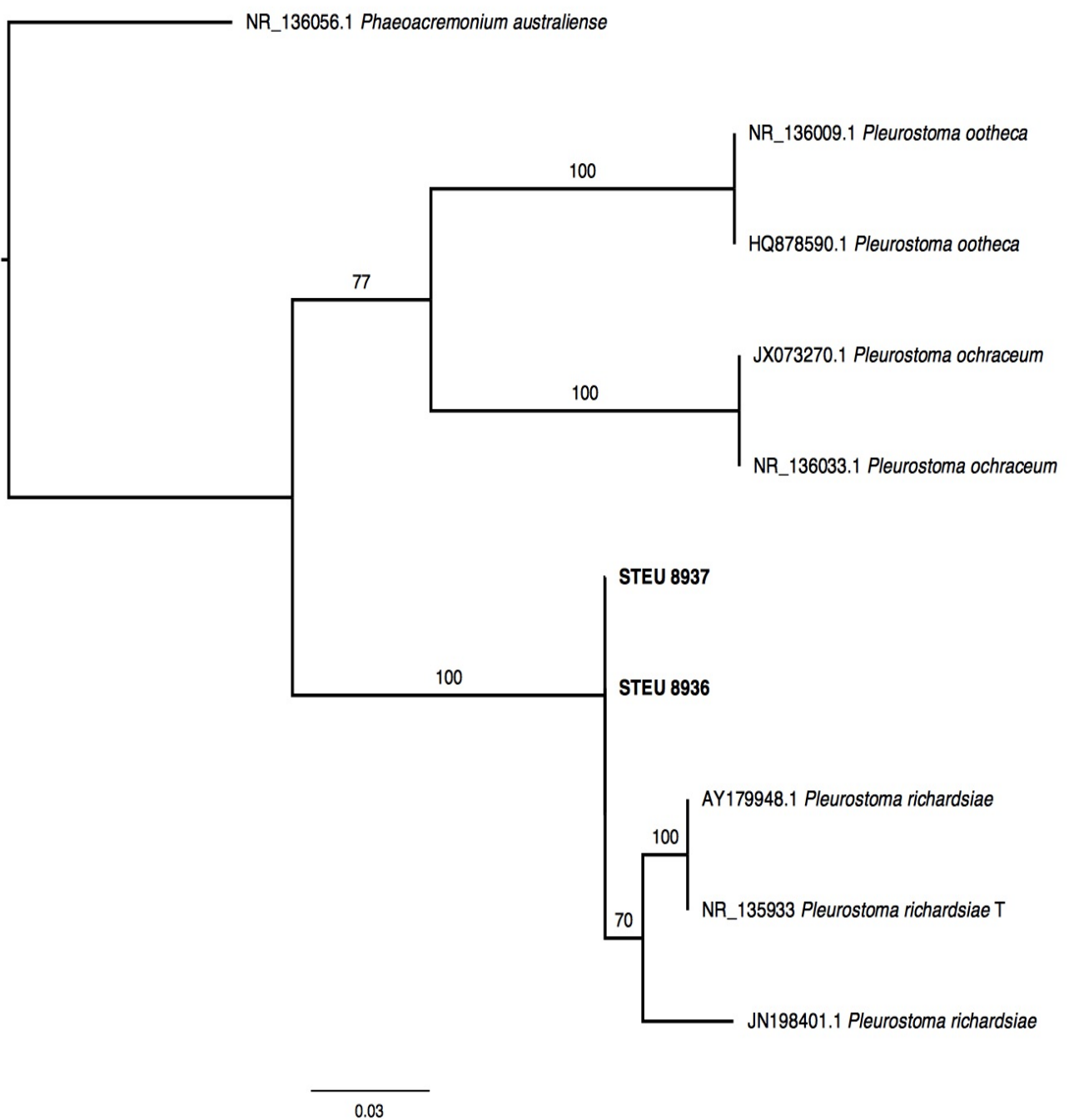
Figure 19. Maximum likelihood phylogenetic tree of *Paraphoma* species based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Paraboeremia putaminum* was used as outgroup and the isolates obtained in this study are indicated in bold.

Figure 20. Maximum likelihood phylogenetic tree of *Phaeoacremonium* species based on β-tubulin sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Calosphaeria africana* and *Jattaea algeriensis* were used as outgroup and the isolates obtained in this study are indicated in bold.

Figure 21. Maximum likelihood phylogenetic tree of *Pleurostoma* species based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Phaeoacremonium australiense* was used as outgroup and the isolates obtained in this study are indicated in bold.
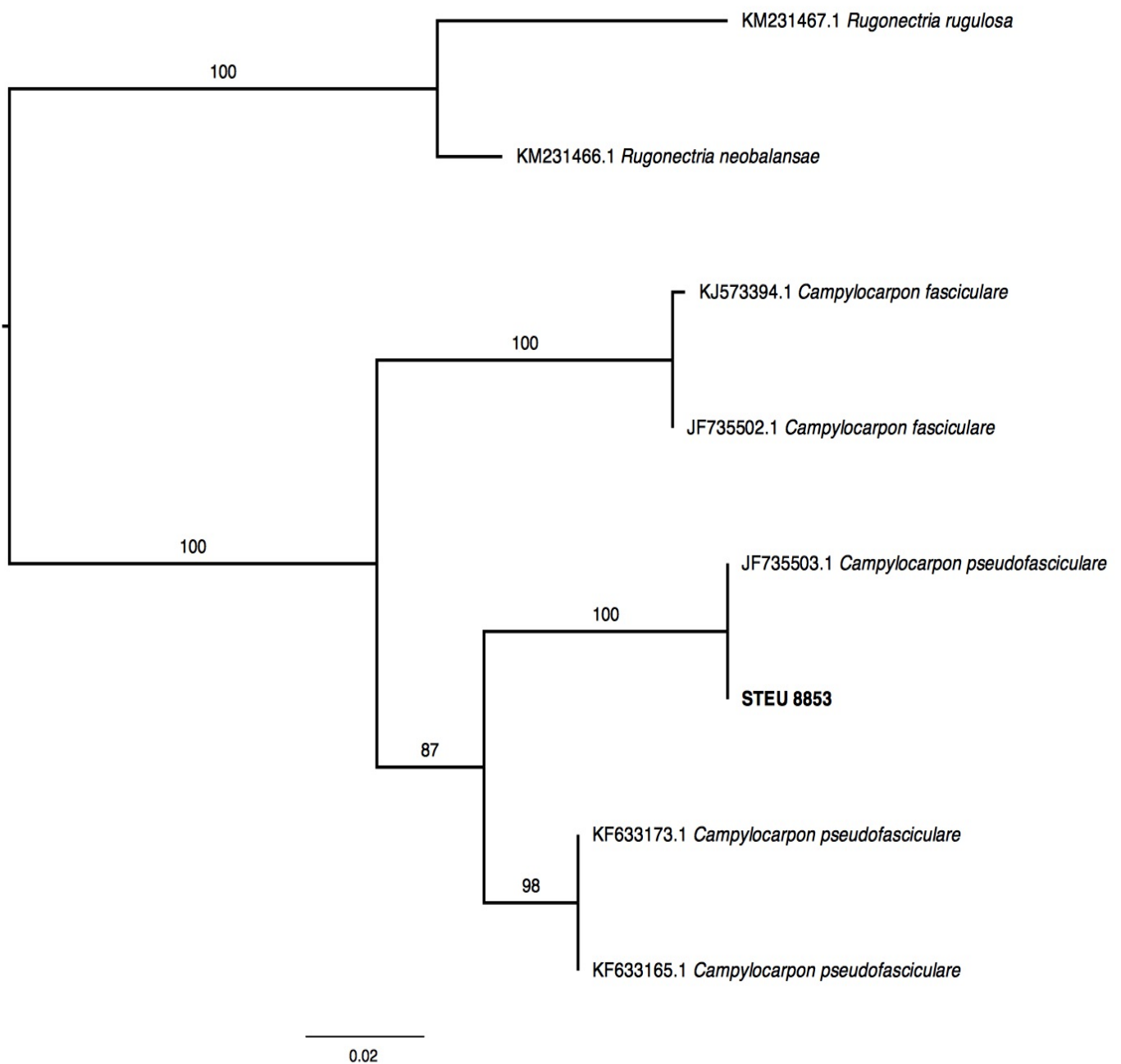
Figure 22. Maximum likelihood phylogenetic tree of *Campylocarpon* species based on histone sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Rugonectria rugulosa* and *Rugonectria neobalansae* were used as outgroup and the isolate obtained in this study is indicated in bold.
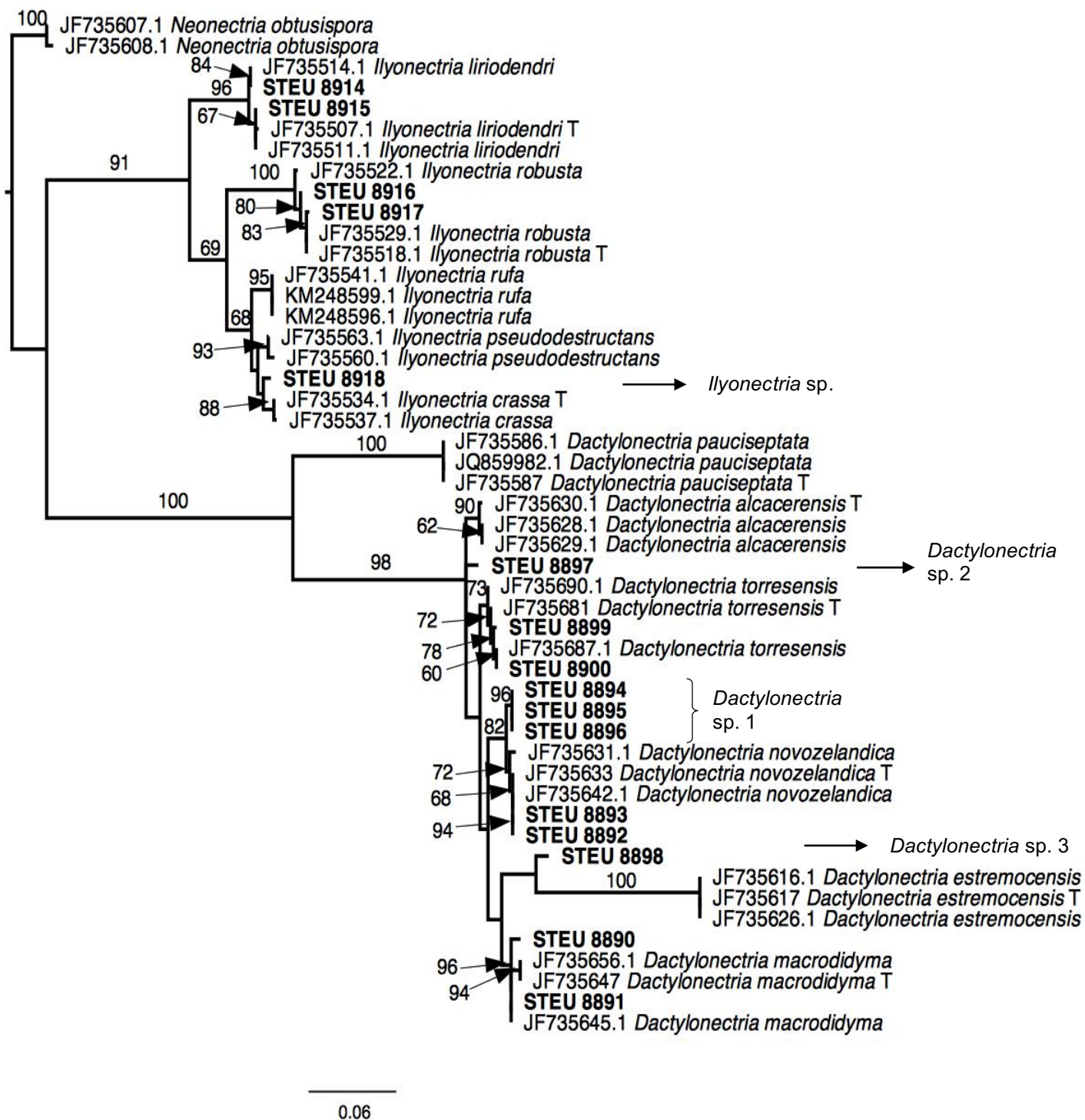
Figure 23. Maximum likelihood phylogenetic tree of "*Cylindrocarpon*"-like fungi which include *Dactylonectria* and *Ilyonectria* species based on histone sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Neonectria obtusispora* was used as outgroup and the isolates obtained in this study are indicated in bold.
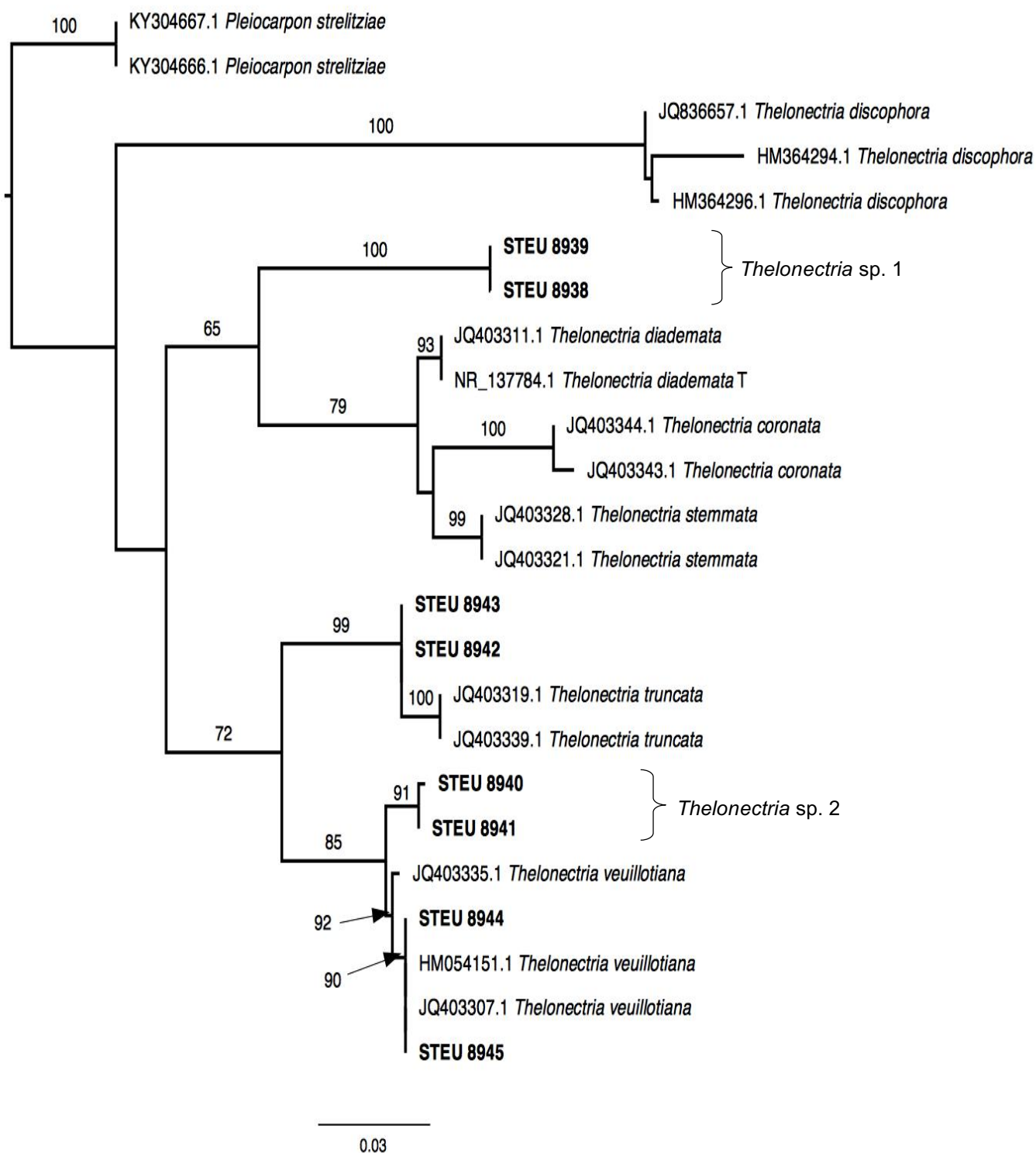
Figure 24. Maximum likelihood phylogenetic tree of *Thelonectria* species based on ITS sequence data. Bootstrap support values were calculated from 100 replicates and bootstrap support of 60% and higher are shown. *Pleiocarpon streilitziae* was used as outgroup and the isolates obtained in this study are indicated in bold.

Figure 25. Sections through ungrafted, rooted rootstock plants. Longitudinal sections through both the top pruning wound and the crown region (A-D). Dark brown streaking symptoms (indicated with an arrow) can be seen at the crown, with the following pathogens isolated: Plum 2 with *Cadophora* sp. (A), Plum 2 with *Cadophora luteo-olivaceae* (B), Plum 1 with *Dactylonectria macrodidyma* (C) and Plum 1 with *Coprinellus flocculosus* (D). Transverse sections through the rootstocks with dark brown necrosis in the rootstock of Plum 2 caused by *Cadophora luteo-olivaceae* (E) and spotting in Plum 1 caused by *Dactylonectria torresensis* (F). Tip dieback at the top pruning wound of a Nectarine 1 plant (G) from which *Collophorina rubra* was isolated.
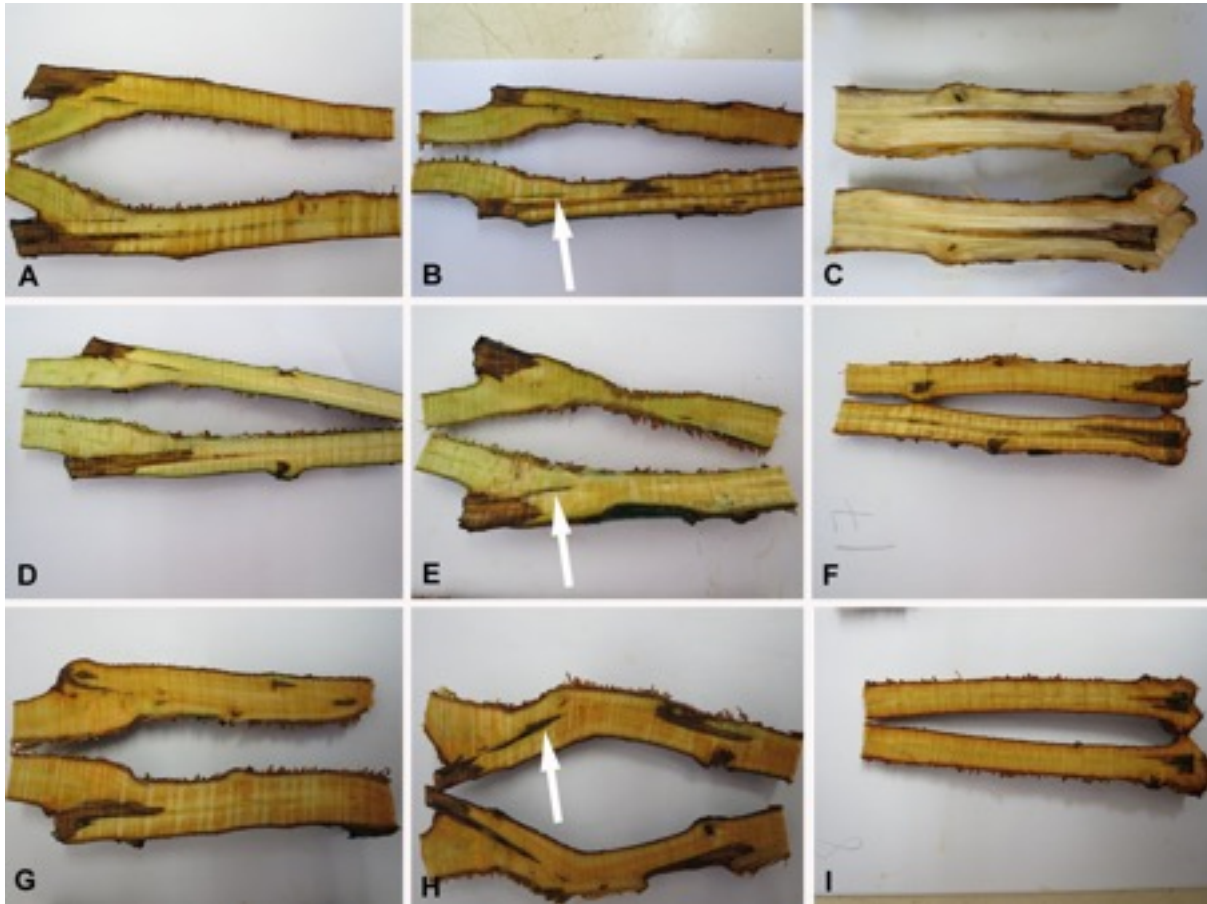
Figure 26. Brown wood discolouration associated with the pruning wound, bud union and crown of stone fruit nursery trees and the associated fungal species isolated: *Dactylonectria* species from the pruning wound with *Dactylonectria novozelandica*, *Dactylonectria* sp.1 and *D. torresensis* (A), bud union with *D. novozelandica*, *Dactylonectria* sp.1, *D. macrodidyma* (B), from the crown of the rootstock *D. novozelandica*, *D. torresensis* (C); only *Cadophora luteo-olivacea* were isolated from the pruning wound (D), bud union (E), crown of the rootstock (F); only *Diplodia seriata* from the pruning wound (G), bud union (H) and together with *D. macrodidyma* from the crown of the rootstock (I). White arrows indicate discolouration at bud union.
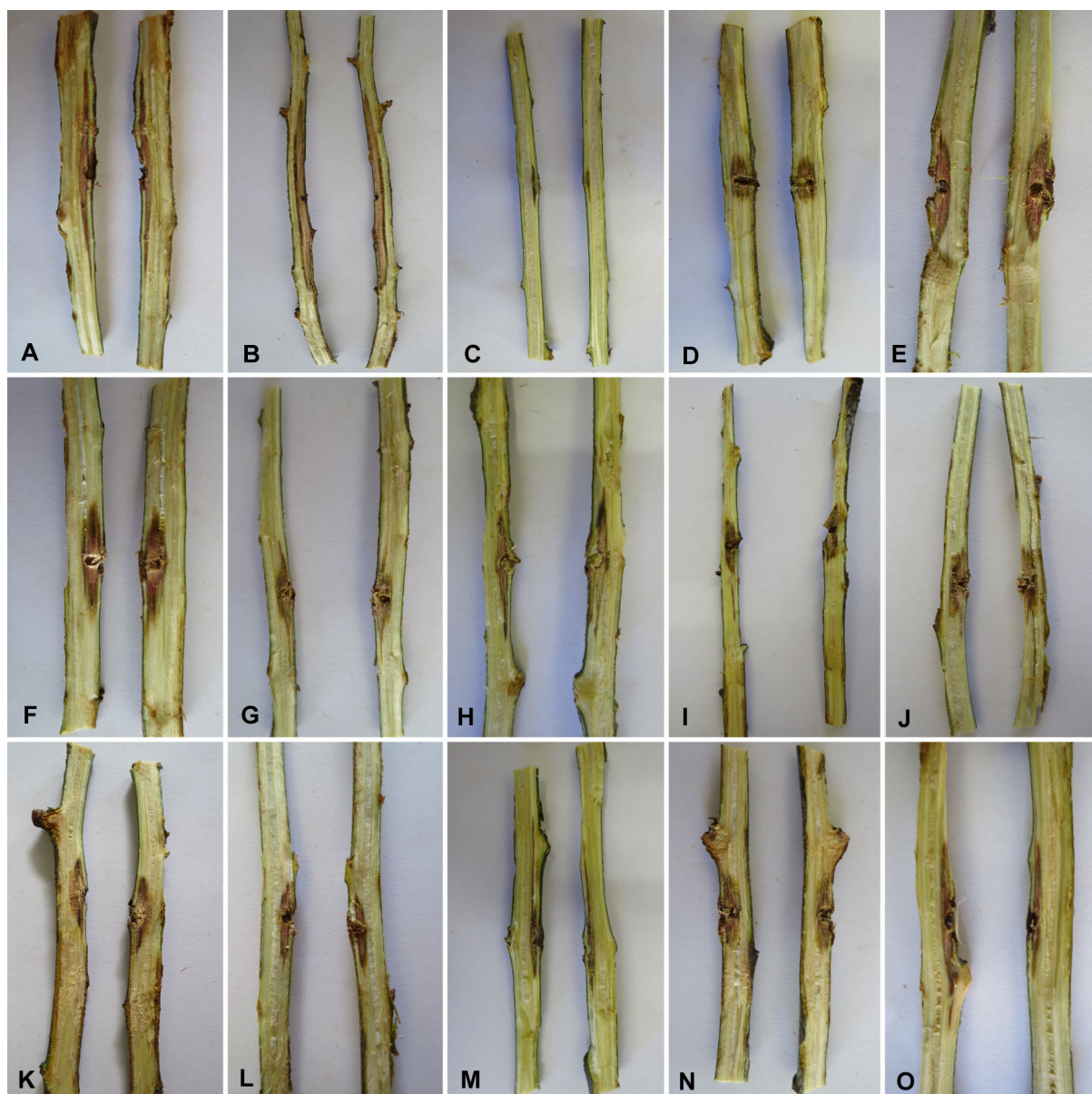
Figure 27. Two- to three-year-old plum shoots (Orchard 1) cut longitudinally to show lesions formed 4 months after inoculation by *Diplodia seriata* STEU 8846 (A), *Lasiodiplodia theobromae* STEU 8849 (B), *Dothiorella moneti* STEU 8847 (C), *Dothiorella viticola* STEU 8848 (D), *Collophorina paarla* STEU 8866 (E), *Collophorina rubra* STEU 8868 (F), *Coniochaeta* sp. 1 STEU 8873 (G), *Coniochaeta* sp. 2 STEU 8875 (H), *Coniochaeta hoffmannii* STEU 8869 (I), *Coniochaeta velutina* STEU 8879 (J), *Cadophora* sp. 2 STEU 8865 (K), *Cadophora novi-eboraci* STEU 8860 (L), *Cadophora gregata* STEU 8855 (M), *Cadophora malorum* STEU 8858 (N), *Cadophora luteo-olivacea* STEU 8857 (O).
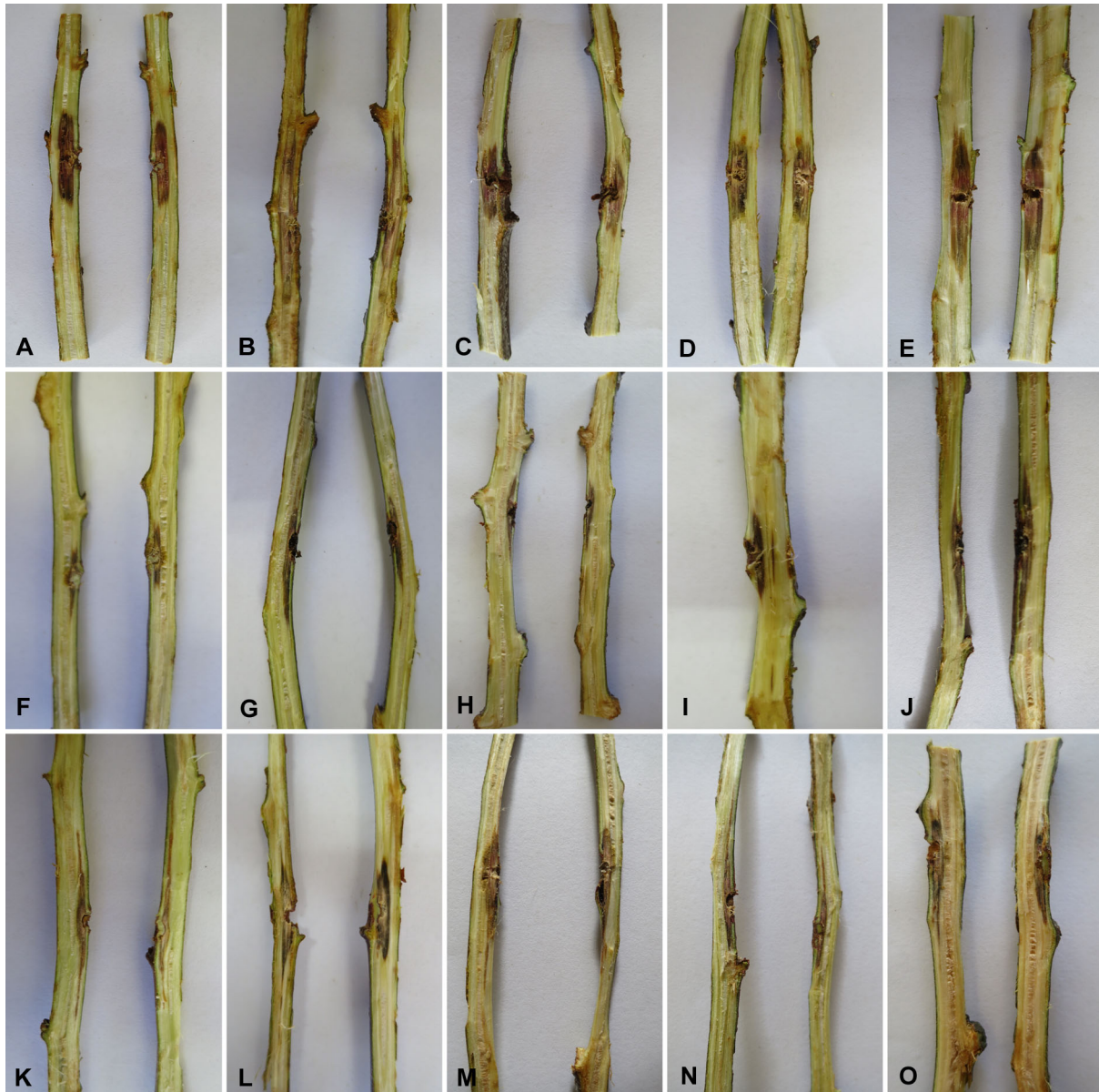
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Figure 28. Two- to three-year-old plum shoots (Orchard 1) cut longitudinally to show lesions formed 4 months after inoculation by *Biscogniauxia* sp. STEU 8843 (A), *Biscogniauxia mediterranea* STEU 8842 (B), *Cytospora leucostoma* STEU 8882 (C), *Cytospora* sp. 1 STEU 8883 (D), *Cytospora* sp. 2 STEU 8887 (E), *Cytospora austromontana* STEU 8880 (F), *Valsa sordida* STEU 8889 (G), *Diaporthe aspalathi* STEU 8903 (H), *Diaporthe foeniculina* STEU 8904 (I), *Diaporthe ambigua* STEU 8902 (J), *Didymella pomorum* STEU 8909 (K), *Didymosphaeria rubi-ulmifolii* STEU 8910 (L), *Didymosphaeria variabile* STEU 8912 (M), *Paraphaeosphaeria neglecta* STEU 8921 (N), *Paraphaeosphaeria sporulosa* STEU 8922 (O).
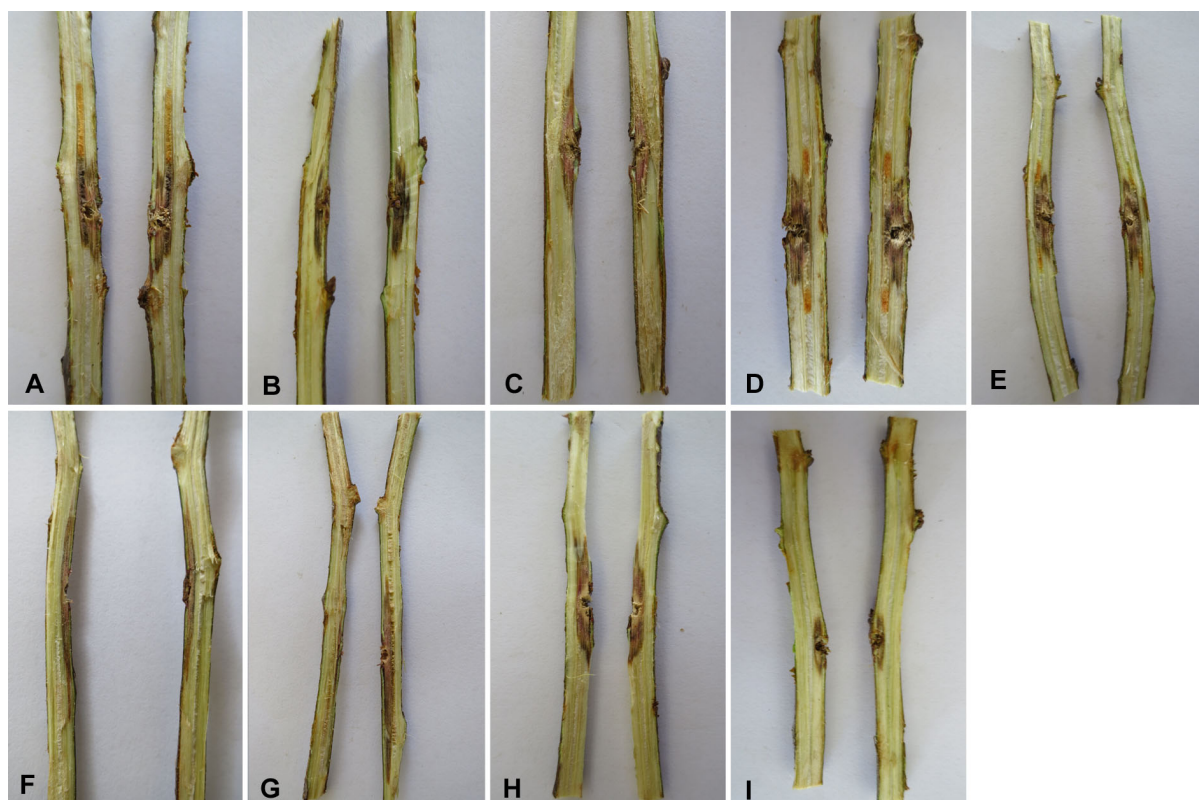
Figure 29. Two- to three-year-old plum shoots (Orchard 1) cut longitudinally to show lesions formed 4 months after inoculation by *Eutypa leptoplaca* STEU 8905 (A), *Eutypella* sp. STEU 8906 (B), *Paraphoma chrysanthemicola* STEU 8924 (C), *Phaeoacremonium parasiticum* STEU 8934 (D), *Phaeoacremonium iranianum* STEU 8929 (E), *Pleurostoma richardsiae* STEU 8936 (F), *Schizophyllum commune* STEU 8841 (G), *Trucatella angustata* STEU 8834 (H), Control (PDA) (I).

ADDENDUM A

Table 1. Ascomycetes associated with dieback or canker on *Prunus* spp. and their worldwide distribution.

Taxonomic group	Causal organism*	Host	Country	Reference
Botryosphaeriaceae	*Aplosporella indica* D.K. Agarwal, Chowdhry & A.K. Sarbhoy	*Prunus domestica*	India	Agarwal *et al.*, 1992
	Aplosporella phyllanthina Syd.	*Prunus domestica*	India	Agarwal *et al.*, 1992
	Aplosporella pruni McAlpine	*Prunus armeniaca*	Australia	McAlpine, 1902
	Aplosporella prunicola Damm & Crous	*Prunus persica* var. *nucipersica*	South Africa	Damm *et al.*, 2007b
	Botryosphaeria dothidea (Moug.) Ces. & De Not.	*Prunus amygdalus*	California	English *et al.*, 1975
		Prunus armeniaca	China, Japan	Kobayashi, 2007; Li and Zhuang, 2007
		Prunus avium	Korea	Cho and Shin, 2004
		Prunus communis	Japan	Slippers *et al.*, 2007
		Prunus domestica	Spain	Roca *et al.*, 2013
		Prunus dulcis	California, Spain	Michailides, 1991; Gramaje *et al.*, 2012
		Prunus jamasakura	Japan	Kobayashi, 2007
		Prunus lannesiana	Japan	Kobayashi, 2007
		Prunus mume	Japan, Taiwan	Kobayashi, 2007; Ko *et al.*, 2011
		Prunus nigra	Kentucky, New Zealand	Flowers *et al.*, 2003; Slippers *et al.*, 2004
		Prunus pendula	Japan	Kobayashi, 2007
		Prunus persica	Alabama, Australia, China, Florida, Georgia, Japan, Louisiana, South Africa, Taiwan, Tennessee, Texas, Worldwide	Britton & Hendrix, 1982; Reilly, 1982; Crous *et al.*, 2000; Cunnington *et al.*, 2007; Slippers *et al.*, 2007; Ko *et al.*, 2011; Gramaje *et al.*, 2012; Tang *et al.*, 2012

* Current name given, disease name given in brackets if known.

Table 1. Continue.

Taxonomic group	Causal organism*	Host	Country	Reference
		Prunus persica var. *vulgaris*	Japan, Korea	Cho and Shin, 2004; Kobayashi, 2007
		Prunus sargentii	Japan	Kobayashi, 2007
		Prunus serotina	Georgia	Hanlin, 1963
		Prunus serrulata	China, Georgia	Hanlin, 1963; Yan *et al*., 2016
		Prunus serrulata var. *spontanea*	Korea	Cho and Shin, 2004
		Prunus sp.	Columbia, Japan, Portugal, Switzerland, United States	Slippers *et al*., 2004, 2007; Kobayashi, 2007; Gramaje *et al*., 2012; Zhu *et al*., 2018
	Diplodia africana (Tuck.) Matzer, H. Mayrhofer & Rambold	*Prunus persica*	South Africa, California, China, Turkey, Uruguay, Worldwide	Damm *et al*., 2007a; Wang *et al*., 2011; Inderbitzin *et al*., 2010; Gramaje *et al*., 2012; Endes *et al*., 2016; Sessa *et al*., 2016
	Diplodia mutila (Fr.) Mont.	*Prunus salicina*	South Africa	Damm *et al*., 2007a
		Prunus laurocerasus	Montenegro, Serbia	Zlatković *et al*., 2018
		Prunus padus	United Kingdom	Dennis, 1986
	Diplodia pinea (Desm.) J. Kickx f.	*Prunus persica*	South Africa	Damm *et al*., 2007a
	Diplodia rosulata Gure, Slippers & Stenlid	*Prunus africana*	Ethiopia, Uruguay	Gure *et al*., 2005; Pérez *et al*., 2010
	Diplodia seriata De Not.	*Prunus persica* var. *nucipersica*	South Africa	Damm *et al*., 2007a
		Prunus salicina	South Africa	Damm *et al*., 2007a; Slippers *et al*., 2007
		Prunus armeniaca	South Africa	Damm *et al*., 2007a

* Current name given, disease name given in brackets if known.

Table 1. Continue.

Taxonomic group	Causal organism*	Host	Country	Reference
		Prunus persica	South Africa	Damm *et al.*, 2007a
		Prunus cerasus	Serbia	Zlatković *et al.*, 2018
		Prunus domestica	Bulgaria	Phillips *et al.*, 2012
		Prunus dulcis	California, United States	Inderbitzin *et al.*, 2010; Gramaje *et al.*, 2012
		Prunus laurocerasus	Italy	Quaglia *et al.*, 2014
		Prunus sp.	United States	Gramaje *et al.*, 2012
	Dothiorella sarmentorum (Fr.) A.J.L. Phillips, A. Alves & J. Luque	*Prunus armeniaca*	Europe, North America	Gramaje *et al.*, 2012
		Prunus dulcis	California, United States	Inderbitzin *et al.*, 2010; Gramaje *et al.*, 2012
		Prunus sp.	North America	Gramaje *et al.*, 2012
	Dothiorella viticola A.J.L. Phillips & J. Luque	*Prunus persica* var. *nucipersica*	South Africa	Damm *et al.*, 2007a
		Prunus salicina	South Africa	Damm *et al.*, 2007a
		Prunus persica	South Africa	Jami *et al.*, 2017
	Lasiodiplodia plurivora Damm & Crous	*Prunus salicina*	South Africa	Damm *et al.*, 2007a
	Lasiodiplodia theobromae (Pat.) Griffon & Maubl.	*Prunus domestica*	California	Inderbitzin *et al.*, 2010
		Prunus dulcis	California	Chen *et al.*, 2013
		Prunus persica	China, Turkey, Worldwide	Wang *et al.*, 2011; Gramaje *et al.*, 2012; Endes *et al.*, 2016

* Current name given, disease name given in brackets if known.

Table 1. Continue.

Taxonomic group	Causal organism*	Host	Country	Reference
		Prunus sp.	United States	Gramaje *et al.*, 2012
	Macrophomina phaseolina	*Prunus armeniaca*	Australia	Cook and Dubé, 1989
	(Tassi) Goid.	*Prunus avium*	Greece	Holevas *et al.*, 2000
		Prunus dulcis	Australia, California, United States	Inderbitzin *et al.*, 2010; Coutinho *et al.*, 2018
		Prunus persica	Australia, Florida	Alfieri Jr. *et al.*, 1984; Cook and Dubé, 1989
	Neofusicoccum australe	*Prunus salicina*	South Africa	Damm *et al.*, 2007a
	(Slippers, Crous & M.J. Wingf.)	*Prunus persica*	South Africa	Damm *et al.*, 2007a
	Crous, Slippers & A.J.L.	*Prunus dulcis*	South Africa	Slippers *et al.*, 2007
	Phillips	*Prunus domestica*	South Africa	Slippers *et al.*, 2007
		Prunus armeniaca	South Africa	Damm *et al.*, 2007a
	Neofusicoccum mediterraneum Crous, M.J. Wingf. & A.J.L. Phillips	*Prunus dulcis*	California, United States	Inderbitzin *et al.*, 2010; Gramaje *et al.*, 2012
	Neofusicoccum nonquaesitum Inderb., Trouillas, R.M. Bostock & Michailides	*Prunus dulcis*	California, United States	Inderbitzin *et al.*, 2010; Gramaje *et al.*, 2012
	Neofusicoccum parvum	*Prunus cerasoides*	Thailand	Trakunyingcharoen *et al.*, 2015
	(Pennycook & Samuels)	*Prunus dulcis*	California, Spain, United States	Inderbitzin *et al.*, 2010; Gramaje *et al.*, 2012
	Crous, Slippers & A.J.L. Phillips	*Prunus laurocerasus*	Montenegro, Serbia	Zlatković *et al.*, 2018

* Current name given, disease name given in brackets if known.

Table 1. Continue.

Taxonomic group	Causal organism*	Host	Country	Reference
		Prunus persica	Australia, China, Greece, Uruguay	Sakalidis *et al.*, 2013; Sessa *et al.*, 2016
		Prunus persica var. *nucipersica*	Greece	Thomidis *et al.*, 2011
	Neofusicoccum ribis (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips	*Prunus* sp.	United States	Gramaje *et al.*, 2012
	Neofusicoccum vitifusiforme (Van Niekerk & Crous) Crous, Slippers & A.J.L. Phillips	*Prunus salicina*	South Africa	Damm *et al.*, 2007a
		Prunus persica	South Africa	Damm *et al.*, 2007a
	Sphaeropsis peckii Sacc.	*Prunus armeniaca*	North Dakota	Brenckle, 1918
		Prunus sp.	United States	Gramaje *et al.*, 2012
Calosphaeriaceae	*Calosphaeria africana* Damm & Crous	*Prunus armeniaca*	South Africa	Damm *et al.*, 2008a
	Jattaea mookgoponga Damm & Crous	*Prunus persica* var. *nucipersica*	South Africa	Damm *et al.*, 2008a
	Jattaea prunicola Damm & Crous	*Prunus salicina*	South Africa	Damm *et al.*, 2008a
Coniochaetaceae	*Coniochaeta africana* Damm & Crous	*Prunus salicina*	South Africa	Damm *et al.*, 2010
	Coniochaeta prunicola Damm & Crous	*Prunus salicina*	South Africa	Damm *et al.*, 2010
		Prunus armeniaca	South Africa	Damm *et al.*, 2010

* Current name given, disease name given in brackets if known.

Table 1. Continue.

Taxonomic group	Causal organism*	Host	Country	Reference
	Coniochaeta velutina (Fuckel) Cooke	*Prunus salicina*	South Africa	Damm *et al.*, 2010
		Prunus armeniaca	South Africa	Damm *et al.*, 2010
Diaporthaceae	*Apiognomonia erythrostoma* (Pers.) Höhn.	*Prunus dulcis*	Worldwide	Gramaje *et al.*, 2012
		Prunus sp.	Worldwide	Gramaje *et al.*, 2012
	Diaporthe ambigua Nitschke	*Prunus salicina*	South Africa	Smit *et al.*, 1996
		Prunus armeniaca	California	Lawrence *et al.*, 2015
		Prunus sp.	South Africa	Santos *et al.*, 2017
	Diaporthe amygdali (Delacr.) Udayanga, Crous & K.D. Hyde (Constriction canker)	*Prunus dulcis*	California, Greece, Hungary, Italy, Portugal, Spain, United States, Worldwide	Adaskaveg *et al.*, 1999; Farr *et al.*, 1999; Diogo *et al.*, 2010; Gramaje *et al.*, 2012; Santos *et al.*, 2017; Varjas *et al.*, 2017
		Prunus amygdalus	China	Santos *et al.*, 2017
		Prunus armeniaca	China	Santos *et al.*, 2017
		Prunus persica	China, France, Georgia, Greece, Japan, Portugal, South Africa, United States	Gomes *et al.*, 2013; Santos *et al.*, 2017
		Prunus persica var. *vulgaris*	Japan	Santos *et al.*, 2017
		Prunus salicina	China, South Africa	Santos *et al.*, 2017
		Prunus salicina var. *corlata*	China	Santos *et al.*, 2017

* Current name given, disease name given in brackets if known.

Table 1. Continue.

Taxonomic group	Causal organism*	Host	Country	Reference
		Prunus sp.	United States	Santos *et al.*, 2017
	Diaporthe foeniculina (Sacc.)	*Prunus dulcis*	Portugal, California	Diogo *et al.*, 2010; Chen *et al.*, 2014
	Udayanga & Castl.	*Prunus amygdalus*	Italy	Santos *et al.*, 2017
		Prunus virginiana	Worldwide	English and Davis, 1965
	Diaporthe perniciosa Marchal	*Prunus dulcis*	Worldwide	Gramaje *et al.*, 2012
	& É.J. Marchal *(*Fruit rot) (Bark	*Prunus cerasus*	Bulgaria	Stoykov and Denchev, 2006
	canker)	*Prunus domestica*	Bulgaria	Stoykov and Denchev, 2006
		Prunus mahaleb	Canada	Ginns, 1986
		Prunus persica	New York, Portugal, Worldwide	Rosenberger and Burr, 1982; Gramaje *et al.*, 2012; Farr and Rossman, 2018
		Prunus sp.	Cyprus, Lithuania, Canada, Poland, Yugoslavia, New Zealand	Georghiou and Papadopoulos, 1957; Ginns, 1986; Pennycook, 1989; Garić and Arsenijević, 1990; Valiuškaitė, 2002; Mułenko *et al.*, 2008
	Diaporthe pruni Ellis & Everh.	*Prunus dulcis*	Worldwide	Gramaje *et al.*, 2012
		Prunus sp.	Worldwide, Canada, Lowa	Gilman and Archer, 1929; Ginns, 1986; Gramaje *et al.*, 2012
		Prunus hortulana	Lowa	Gilman and Archer, 1929
		Prunus serotina	Lowa	Gilman and Archer, 1929
		Prunus virginiana	Canada	Farr and Rossman, 2018
		Prunus x *yedoensis*	Japan	Kobayashi, 2007
	Phomopsis padina (Sacc.)	*Prunus avium*	Washington	Shaw, 1973
	Died.	*Prunus dulcis*	Worldwide	Gramaje *et al.*, 2012

* Current name given, disease name given in brackets if known.

Table 1. Continue.

Taxonomic group	Causal organism*	Host	Country	Reference
		Prunus padus	Scotland	Kirk and Spooner, 1984
		Prunus persica	Worldwide	Gramaje *et al.*, 2012
	Phomopsis parabolica Petr.	*Prunus dulcis*	Worldwide	Gramaje *et al.*, 2012
		Prunus persica	Worldwide	Gramaje *et al.*, 2012
	Phomopsis prunorum (Cooke) Grove	*Prunus domestica*	Central Asia, Washington	Shaw, 1973; Farr and Rossman, 2018
		Prunus dulcis	Worldwide	Gramaje *et al.*, 2012
		Prunus sp.	Worldwide	Gramaje *et al.*, 2012
	Phomopsis ribatejana Sousa da Câmara	*Prunus dulcis*	Worldwide	Gramaje *et al.*, 2012
		Prunus sp.	Worldwide	Gramaje *et al.*, 2012
Diatrypaceae	*Cryptovalsa ampelina* (Nitschke) Fuckel	*Prunus armeniaca*	California, South Africa, United States	Trouillas *et al.*, 2010; Moyo *et al.*, 2018
		Prunus salicina	South Africa	Moyo *et al.*, 2018
		Prunus sp.	United States, South Africa	Damm *et al.*, 2009; Gramaje *et al.*, 2012
	Diatrype oregonensis (Wehm.) Rappaz	*Prunus armeniaca*	California, United States	Trouillas *et al.*, 2010
	Eutypa cremea Moyo, Halleen, L. Mostert	*Prunus armeniaca*	South Africa	Moyo *et al.*, 2018
		Prunus salicina	South Africa	Moyo *et al.*, 2018
	Eutypa lata (Pers.) Tul. & C. Tul.	*Prunus americana*	North Dakota, Switzerland	Brenckle, 1917; Rappaz, 1987
		Prunus amygdalus	Australia	Rappaz, 1987
		Prunus armeniaca	Australasia, Australia, California, Europe, Greece, New Zealand, North America, South Africa, Worldwide	Cook and Dubé, 1989; Pennycook, 1989; Carter, 1991; Holevas *et al.*, 2000; Travadon and Baumgartner, 2015; Moyo *et al.*, 2018

* Current name given, disease name given in brackets if known.

Table 1. Continue.

Taxonomic group	Causal organism*	Host	Country	Reference
		Prunus avium	California, Europe, Switzerland, Worldwide	Rappaz, 1987; Carter, 1991; Munkvold and Marois, 1991, 1995
		Prunus demissa	North America	Carter, 1991
		Prunus domestica	Australasia, Australia, Europe, North America, South Africa	Cook and Dubé, 1989; Carter, 1991
		Prunus dulcis	Australasia, Australia, Europe, Worldwide	Carter, 1982, 1991; Cook and Dubé, 1989
		Prunus persica	Australasia, Australia, Europe	Cook and Dubé, 1989; Carter, 1991
		Prunus salicina	Australasia, Australia, South Africa, Worldwide	Carter, 1982, 1991; Cook and Dubé, 1989; Moyo *et al.*, 2018
		Prunus sp.	Australia, Rhode Island	Cunnington, 2003; Goos, 2010
		Prunus spinosa	Australasia, Poland, Switzerland	Rappaz, 1987; Carter, 1991; Mułenko *et al.*, 2008
	Eutypa leptoplaca (Durieu & Mont.) Rappaz	*Prunus armeniaca*	Argenitina	Carmarán *et al.*, 2009
	Eutypella citricola Speg.	*Prunus americana*	South Africa	Moyo *et al.*, 2018
		Prunus salicina	South Africa	Moyo *et al.*, 2018
	Eutypella microtheca Trouillas, W.M. Pitt & Gubler	*Prunus salicina*	South Africa	Moyo *et al.*, 2018
		Prunus armeniaca	South Africa	Moyo *et al.*, 2018
	Eutypella prunastri (Pers.) Sacc.	*Prunus avium*	Austria, Switzerland, Worldwide	Rappaz, 1987; Gramaje *et al.*, 2012; Farr and Rossman, 2018
		Prunus divaricata	Ukraine	Dudka *et al.*, 2004

* Current name given, disease name given in brackets if known.

Table 1. Continue.

Taxonomic group	Causal organism*	Host	Country	Reference
		Prunus dulcis	Worldwide	Gramaje *et al.*, 2012
		Prunus salicina	Worldwide	Gramaje *et al.*, 2012
		Prunus sp.	Italy, Sweden	Venturella, 1991; Eriksson, 2014
		Prunus spinosa	Denmark, England, Poland, United Kingdom, Worldwide	Munk, 1957; Scheuer and Chlebicki, 1997; Gramaje *et al.*, 2012; Farr and Rossman, 2018
Didymosphaeriaceae	*Didymosphaeria rubi-ulmifolii* Ariyaw., Camporesi & K.D. Hyde	*Prunus persica* var. *nucipersica*	South Africa	Damm *et al.*, 2008c
		Prunus persica	South Africa	Damm *et al.*, 2008c
		Prunus salicina	South Africa	Damm *et al.*, 2008c
	Didymosphaeria variabile (Riccioni, Damm, Verkley & Crous) Ariyaw. & K.D. Hyde	*Prunus salicina*	South Africa	Damm *et al.*, 2008c
		Prunus persica	South Africa	Damm *et al.*, 2008c
	Pseudocamarosporium africanum (Damm, Verkley & Crous) Crous	*Prunus persica*	South Africa	Damm *et al.*, 2008c
Phaeomoniellaceae	*Aequabiliella effuse* (Damm & Crous) Crous	*Prunus persica*	South Africa	Damm *et al.*, 2010
		Prunus salicina	South Africa	Damm *et al.*, 2010
	Celerioriella dura (Damm & Crous) Crous	*Prunus salicina*	South Africa	Damm *et al.*, 2010

* Current name given, disease name given in brackets if known.

Table 1. Continue.

Taxonomic group	Causal organism*	Host	Country	Reference
	Celerioriella prunicola (Damm & Crous) Crous	*Prunus salicina*	South Africa	Damm *et al.*, 2010
	Minutiella tardicola (Damm & Crous) Crous	*Prunus armeniaca*	South Africa	Damm *et al.*, 2010
	Neophaeomoniella zymoides (Hyang B. Lee, J.Y. Park, Summerb. & H.S. Jung) Crous	*Prunus salicina*	South Africa	Damm *et al.*, 2010
Togniniaceae	*Phaeoacremonium africanum* (Damm, L. Mostert & Crous) Gramaje, L. Mostert & Crous	*Prunus armeniaca*	South Africa	Damm *et al.*, 2008b
	Pheaoacremonium alvesii L. Mostert, Summerb. & Crous	*Prunus persica*	South Africa	Spies *et al.*, 2018
	Phaeoacremonium australiense L. Mostert, Summerb. & Crous	*Prunus salicina*	South Africa, Australia	Damm *et al.*, 2008b
	Phaeoacremonium fraxinopennsylvanicum (T.E. Hinds) Gramaje, L. Mostert & Crous	*Prunus salicina*	South Africa	Damm *et al.*, 2008b; Gramaje *et al.*, 2015
	Phaeoacremonium fuscum L. Mostert, Damm & Crous	*Prunus salicina*	South Africa	Damm *et al.*, 2008b

* Current name given, disease name given in brackets if known.

Table 1. Continue.

Taxonomic group	Causal organism*	Host	Country	Reference
	Phaeoacremonium griseo-olivaceum (Damm, L. Mostert & Crous) Gramaje, L. Mostert & Crous	*Prunus armeniaca*	South Africa	Damm *et al.*, 2008b; Gramaje *et al.*, 2015
	Phaeoacremonium griseorubrum L. Mostert, Summerb. & Crous	*Prunus salicina*	South Africa	Damm *et al.*, 2008b
		Prunus persica	South Africa	Spies *et al.*, 2018
	Phaeoacremonium iranianum L. Mostert, Gräfenhan, W. Gams & Crous	*Prunus salicina*	South Africa	Damm *et al.*, 2008b; Spies *et al.*, 2018
		Prunus armeniaca	South Africa	Damm *et al.*, 2008b
		Prunus dulcis	Spain	Gramaje *et al.*, 2012
		Prunus persica var. *nucipersica*	South Africa	Spies *et al.*, 2018
	Phaeoacremonium minimum (Tul. & C. Tul.) Gramaje, L. Mostert & Crous	*Prunus salicina*	South Africa	Damm *et al.*, 2008b
		Prunus persica	South Africa	Damm *et al.*, 2008b
		Prunus pennsylvanica	South Africa, United States	Hausner *et al.*, 1992; Gramaje *et al.*, 2015
		Prunus armeniaca	South Africa, Iran	Mostert *et al.*, 2006; Damm *et al.*, 2008b; Arzanlou *et al.*, 2014
		Prunus dulcis	South Africa, Spain	Marín-Terrazas *et al.*, 2016; Spies *et al.*, 2018
	Phaeoacremonium pallidum Damm, L. Mostert & Crous	*Prunus armeniaca*	South Africa	Damm *et al.*, 2008b

* Current name given, disease name given in brackets if known.

Table 1. Continue.

Taxonomic group	Causal organism*	Host	Country	Reference
	Phaeoacremonium parasiticum (Ajello, Georg & C.J.K. Wang) W. Gams, Crous & M.J. Wingf.	*Prunus armeniaca* *Prunus avium*	South Africa, Tunisia Greece	Crous *et al.*, 2000; Damm *et al.*, 2008 Gramaje *et al.*, 2015
	Phaeoacremonium prunicola L. Mostert, Damm & Crous	*Prunus salicina*	South Africa	Damm *et al.*, 2008b
	Phaeoacremonium scolyti L. Mostert, Summerb. & Crous	*Prunus persica* var. *nucipersica*	South Africa	Damm *et al.*, 2008b
		Prunus salicina	South Africa	Damm *et al.*, 2008b
		Prunus persica	South Africa	Damm *et al.*, 2008b
		Prunus dulcis	South Africa	Spies *et al.*, 2018
		Prunus domestica	South Africa	Spies *et al.*, 2018
		Prunus armeniaca	South Africa	Damm *et al.*, 2008b
	Phaeoacremonium subulatum L. Mostert, Summerb. & Crous	*Prunus armeniaca* *Prunus salicina*	South Africa South Africa	Damm *et al.*, 2008b Spies *et al.*, 2018
	Phaeoacremonium viticola J. Dupont	*Prunus salicina* *Prunus armeniaca*	South Africa South Africa	Damm *et al.*, 2008b Damm *et al.*, 2008b
Tympanidaceae	*Collophorina africana* (Damm & Crous) Damm & Crous	*Prunus salicina*	South Africa	Damm *et al.*, 2010
	Collophorina hispanica (Gramaje, Armengol & Damm) Damm & Crous	*Prunus dulcis*	California, Iran, Spain	Gramaje *et al.*, 2012; Arzanlou *et al.*, 2016; Holland *et al.*, 2018

* Current name given, disease name given in brackets if known.

Table 1. Continue.

Taxonomic group	Causal organism*	Host	Country	Reference
	Collophorina paarla (Damm & Crous) Damm & Crous	*Prunus salicina*	South Africa	Damm *et al.*, 2010
		Prunus persica	South Africa	Damm *et al.*, 2010
		Prunus dulcis	California	Holland *et al.*, 2018
	Collophorina rubra (Damm & Crous) Damm & Crous	*Prunus persica* var. *nucipersica*	South Africa	Damm *et al.*, 2010
		Prunus salicina	South Africa	Damm *et al.*, 2010
		Prunus persica	South Africa	Damm *et al.*, 2010
		Prunus dulcis	South Africa	Damm *et al.*, 2010
Valsariaceae	*Leucostoma persoonii* (Nitschke) Höhn.	*Prunus armeniaca*	Japan, Michigan, South Africa	Surve-Iyer *et al.*, 1995; Crous *et al.*, 2000; Kobayashi, 2007
		Prunus avium	Bulgaria, California, Japan, Oregon, South Africa	Surve-Iyer *et al.*, 1995; Crous *et al.*, 2000; Kobayashi, 2007; Trouillas *et al.*, 2012; Farr and Rossman, 2018
		Prunus cerasus	Japan, Michigan	Surve-Iyer *et al.*, 1995; Kobayashi, 2007
		Prunus domestica	Brazil, Bulgaria, California, Canada, Michigan, Switzerland	Ginns, 1986; Surve-Iyer *et al.*, 1995; Farr and Rossman, 2018; Sessa *et al.*, 2018
		Prunus dulcis	Italy	Venturella, 1991
		Prunus mume	Japan	Kobayashi, 2007
		Prunus padus	Japan	Kobayashi, 2007
		Prunus pensylvanica	Canada	Ginns, 1986

* Current name given, disease name given in brackets if known.

Table 1. Continue.

Taxonomic group	Causal organism*	Host	Country	Reference
		Prunus persica	Brazil, Bulgaria, Canada, Japan, Michigan, North Carolina, New Jersey, South Africa, United States, Uruguay	Ginns, 1986; Biggs *et al.*, 1992; Surve-Iyer *et al.*, 1995; Crous *et al.*, 2000; Vrandecic *et al.*, 2010; Farr and Rossman, 2018; Sessa *et al.*, 2018
		Prunus persica var. *nucipersica*	Canada, California	Ginns, 1986; Surve-Iyer *et al.*, 1995
		Prunus persica var. *vulgaris*	Japan, Korea	Cho and Shin, 2004; Kobayashi, 2007
		Prunus pseudocerasus	Japan	Kobayashi, 2007
		Prunus salicina	Japan, South Africa	Crous *et al.*, 2000; Kobayashi, 2007
		Prunus serotina	Michigan	Surve-Iyer *et al.*, 1995
		Prunus sp.	Australia, Japan, United Kingdom	Cannon *et al.*, 1985; Cunnington, 2003; Kobayashi, 2007
		Prunus spinosa	Bulgaria, Poland, Ukraine	Dudka *et al.*, 2004; Stoykov and Denchev, 2006; Mułenko *et al.*, 2008
		Prunus virginiana	Canada	Ginns, 1986
	Valsaria insitiva (Tode) Ces. & De Not. (Leucostoma canker)	*Prunus serotina*	Virginia	Ju *et al.*, 1996
		Prunus spinosa	Poland	Mułenko *et al.*, 2008

* Current name given, disease name given in brackets if known.

Table 2. Basidiomycetes associated with wood rot on *Prunus* spp. and their worldwide distribution.

Causal organism*	Host	Country	Reference
Antrodia albida (Fr.) Donk (Brown rot)	*Prunus persica*	South Carolina	Adaskaveg *et al.*, 1993
	Prunus sp.	Japan	Kobayashi, 2007
Armillaria mellea (Vahl) P. Kumm. (White rot)	*Prunus alleghaniensis*	California	Raabe, 1965
	Prunus americana	California, Florida	Alfieri Jr. *et al.*, 1984; French, 1989
	Prunus amygdalus	California	Farr and Rossman, 2018
	Prunus angustifolia	Florida	Alfieri Jr. *et al.*, 1984
	Prunus armeniaca	California, Florida, Japan, Kenya, South Africa, Zimbabwe	Doidge, 1950; Nattrass, 1961; Alfieri Jr. *et al.*, 1984; French, 1989; Whiteside, 1996; Kobayashi, 2007
	Prunus avium	Bulgaria, California, Canada, Greece, South Africa	Doidge, 1950; Ginns, 1986; French, 1989; Zervakis *et al.*, 1998; Farr and Rossman, 2018
	Prunus blireiana	California	French, 1989
	Prunus caroliniana	California	Raabe, 1965
	Prunus cerasifera	California, Canada	Ginns, 1986; French, 1989
	Prunus cerasus	Bulgaria, California, Canada, Greece, Zimbabwe	Ginns, 1986; French, 1989; Whiteside, 1996; Zervakis *et al.*, 1998; Farr and Rossman, 2018
	Prunus davidiana	California	French, 1989
	Prunus domestica	Australia, Brazil, Bulgaria, California, Florida, Hawaii, Kenya, Oregon, Washington	Nattrass, 1961; Simmonds, 1966; Shaw, 1973; Laemmlen and Bega, 1974; Alfieri Jr. *et al.*, 1984; French, 1989; Farr and Rossman, 2018
	Prunus dulcis	California, Greece	Adaskaveg and Ogawa, 1990; Zervakis *et al.*, 1998
	Prunus emarginata	California, Washington	Shaw, 1973; Farr and Rossman, 2018
	Prunus grayana	Japan	Kobayashi, 2007

* Current name given, disease name given in brackets if known.

Table 2. Continue.

Causal organism*	Host	Country	Reference
	Prunus lannesiana	Japan	Kobayashi, 2007
	Prunus lannesiana f. alborosea	Japan	Kobayashi, 2007
	Prunus mahaleb	California, Canada, Greece	Ginns, 1986; French, 1989; Zervakis *et al.*, 1998
	Prunus mume	Japan	Kobayashi, 2007
	Prunus munsoniana	Florida	Alfieri Jr. *et al.*, 1984
	Prunus persica	Australia, Brazil, Bulgaria, California, Florida, France, Greece, Hawaii, Idaho, Kenya, Malawi, Mexico, Missouri, Mississippi, Oklahoma, Oregon, South Carolina, South Africa, Washington, Zimbabwe	Maneval, 1937; Preston, 1945; Doidge, 1950; Parris, 1959; Nattrass, 1961; Corbett, 1964; Simmonds, 1966; Pantidou, 1973; Shaw, 1973; Raabe *et al.*, 1981; Alfieri Jr. *et al.*, 1984; Adaskaveg and Ogawa, 1990; Whiteside, 1996; Chillali *et al.*, 1998; Cox *et al.*, 2006; Elias-Roman *et al.*, 2013; Farr and Rossman, 2018
	Prunus persica var *nectarina*	California	French, 1989
	Prunus persica var *vulgaris*	Japan	Kobayashi, 2007
	Prunus salicina	California, South Africa, Zimbabwe	Doidge, 1950; French, 1989; Whiteside, 1996
	Prunus serotina	Canada, New York	Anderson and Ullrich, 1979; Ginns, 1986
	Prunus sp.	Greece, Japan, Oklahoma	Preston, 1945; Zervakis *et al.*, 1998; Kobayashi, 2007
	Prunus ssiori	Japan	Kobayashi, 2007
	Prunus subhirtella	Japan	Kobayashi, 2007
	Prunus triloba	Scotland	Foister, 1961

* Current name given, disease name given in brackets if known.

Table 2. Continue.

Causal organism*	Host	Country	Reference
	Prunus verecunda	Japan	Kobayashi, 2007
	Prunus virginiana var. demissa	California	Farr and Rossman, 2018
	Prunus x *yedoensis*	Japan	Kobayashi, 2007
Chondrostereum purpureum (Pers.) Pouzar (Silver leaf Disease)	*Prunus armeniaca*	Australia, California, Japan, New Zealand, South Africa	French, 1987; Cook and Dubé, 1989; Crous *et al.*, 2000; Gadgil, 2005; Kobayashi, 2007
	Prunus avium	Australia, Montana, New Zealand	Shaw, 1973; Cook and Dubé, 1989; Gadgil, 2005
	Prunus cerasifera	Australia, New Zealand	Cook and Dubé, 1989; Gadgil, 2005
	Prunus cerasus	Australia	Cook & Dubé, 1989
	Prunus domestica	Australia, California, Canada, New Zealand, Washington	Shaw, 1973; Ginns, 1986; French, 1987; Cook and Dubé, 1989; Gadgil, 2005
	Prunus dulcis	Australia, New Zealand, South Africa	Cook and Dubé, 1989; Crous *et al.*, 2000; Gadgil, 2005
	Prunus emarginata	Canada	Ginns, 1986
	Prunus laurocerasus	Canada	Ginns, 1986
	Prunus lusitanica	New Zealand	Gadgil, 2005
	Prunus mume	Japan	Kobayashi, 2007
	Prunus pensylvanica	Canada	Ginns, 1986
	Prunus persica	Australia, California, Greece, New Zealand, South Africa	French, 1987; Cook and Dubé, 1989; Crous *et al.*, 2000; Holevas *et al.*, 2000; Gadgil, 2005
	Prunus persica var. nectarina	Australia	Cook & Dubé, 1989

* Current name given, disease name given in brackets if known.

Table 2. Continue.

Causal organism*	Host	Country	Reference
	Prunus persica var. *nucipersica*	New Zealand	Gadgil, 2005
	Prunus persica var. *vulgaris*	Japan	Kobayashi, 2007
	Prunus salicina	Australia, Japan, New Zealand, South Africa	Cook & Dubé, 1989; Crous *et al.*, 2000; Gadgil, 2005; Kobayashi, 2007
	Prunus serrulata	New Zealand	Gadgil, 2005
	Prunus sp.	California, Canada, Ireland, Japan, New York, Ukraine	Ginns, 1986; French, 1987; Chamuris, 1988; Dudka *et al.*, 2004; Kobayashi, 2007; Muskett and Malone, 2013
Fomes meliae (Underw.) Murrill (Brown rot)	*Prunus persica*	South Carolina	Adaskaveg *et al.*, 1993
Fomitiporia robusta (P. Karst.) Fiasson & Niemelä (White rot)	*Prunus avium*	California	Adaskaveg and Ogawa, 1990
	Prunus domestica	Kenya	Nattrass, 1961
	Prunus dulcis	California	Adaskaveg and Ogawa, 1990
	Prunus mahaleb	California	Adaskaveg and Ogawa, 1990
	Prunus persica	California, South Carolina	Adaskaveg and Ogawa, 1990; Adaskaveg *et al.*, 1993
	Prunus persica var. *vulgaris*	Japan	Kobayashi, 2007
	Prunus sp.	California	Adaskaveg and Ogawa, 1990
Ganoderma brownii (Murrill) Gilb. (White rot)	*Prunus dulcis*	California	Adaskaveg and Ogawa, 1990
	Prunus persica	California	Adaskaveg and Ogawa, 1990
Irpex lacteus (Fr.) Fr. (White rot)	*Prunus americana*	North dakota, Canada	Brenckle, 1918; Ginns, 1986

* Current name given, disease name given in brackets if known.

Table 2. Continue.

Causal organism*	Host	Country	Reference
	Prunus avium	California	Adaskaveg and Ogawa, 1990
	Prunus mahaleb	California	Adaskaveg and Ogawa, 1990
	Prunus pensylvanica	Canada	Ginns, 1986
	Prunus persica	South Carolina	Adaskaveg *et al.*, 1993
	Prunus sargentii	Japan	Kobayashi, 2007
	Prunus serotina	Canada	Ginns, 1986
	Prunus sp.	California, Canada, Japan	Ginns, 1986; Adaskaveg and Ogawa, 1990; Kobayashi, 2007
Laetiporus sulphureus (Bull.) Murrill (Brown rot)	*Prunus americana*	California, Greece	Adaskaveg and Ogawa, 1990; Zervakis *et al.*, 1998
	Prunus avium	Bulgaria, Germany	Rogers *et al.*, 1999; Farr & Rossman, 2018
	Prunus cerasus	Bulgaria	Farr and Rossman, 2018
	Prunus domestica	Bulgaria, California, Ukraine	Adaskaveg and Ogawa, 1990; Dudka *et al.*, 2004; Farr and Rossman, 2018
	Prunus dulcis	California	Adaskaveg and Ogawa, 1990
	Prunus insititia	Switzerland	Rogers et al., 1999
	Prunus pseudocerasus	Japan	Kobayashi, 2007
	Prunus serotina	Canada	Ginns, 1986
	Prunus sp.	Argentina, California, Canada, China, Japan, Slovakia	Ginns, 1986; Adaskaveg & Ogawa, 1990; Chen, 2002; Kobayashi, 2007; Rajchenberg and Robledo, 2013; Farr and Rossman, 2018
Oxyporus latemarginatus (Durieu & Mont.) Donk (White rot)	*Prunus avium*	California	Adaskaveg and Ogawa, 1990

* Current name given, disease name given in brackets if known.

Table 2. Continue.

Causal organism*	Host	Country	Reference
	Prunus dulcis	California	Adaskaveg and Ogawa, 1990
	Prunus mahaleb	California	Adaskaveg and Ogawa, 1990
	Prunus persica	South Carolina	Adaskaveg *et al.*, 1993
	Prunus sp.	California, Portugal	Adaskaveg and Ogawa, 1990; Farr and Rossman, 2018
Phellinus gilvus (Schwein.) Pat. (White rot)	*Prunus dulcis*	California	Adaskaveg and Ogawa, 1990
	Prunus persica	Australia, California, South Carolina	Cook & Dubé, 1989; Adaskaveg and Ogawa, 1990; Adaskaveg *et al.*, 1993
	Prunus serotina	Rhode Island	Goos, 2010
	Prunus serotina subsp. *virens*	Arizona	Gilbertson *et al.*, 1974
	Prunus serrulata var. *spontanea*	Korea	Cho and Shin, 2004
	Prunus sp.	California	Adaskaveg and Ogawa, 1990
Phellinus robustus (P. Karst.) Bourdot & Galzin (White rot)	*Prunus americana*	California, New Zealand	Cunningham, 1965; Adaskaveg and Ogawa, 1990
	Prunus domestica	California	Adaskaveg and Ogawa, 1990
	Prunus dulcis	Australia, California	Cook & Dubé, 1989; Adaskaveg and Ogawa, 1990
Rhodofomes cajanderi (P. Karst.) B.K. Cui, M.L. Han & Y.C. Dai (Brown rot)	*Prunus americana*	California	Adaskaveg and Ogawa, 1990
	Prunus domestica	California	Adaskaveg and Ogawa, 1990
	Prunus pensylvanica	Canada	Ginns, 1986

* Current name given, disease name given in brackets if known.

Table 2. Continue.

Causal organism*	Host	Country	Reference
Schizophyllum commune Fr. (White rot)	*Prunus armeniaca*	Australia, Bulgaria, California, Canada, Japan, South Africa, Washington	Shaw, 1973; Ginns, 1986; Cook and Dubé, 1989; Adaskaveg and Ogawa, 1990; Crous *et al.*, 2000; Kobayashi, 2007; Farr and Rossman, 2018
	Prunus avium	Bulgaria, Australia, California, Greece, Japan, South Africa	Pantidou, 1973; Cook and Dubé, 1989; Adaskaveg and Ogawa, 1990; Crous *et al.*, 2000; Kobayashi, 2007; Farr and Rossman, 2018
	Prunus cerasus	Bulgaria	Farr and Rossman, 2018
	Prunus davidiana	China	Chen, 2002
	Prunus domestica	Bulgaria, Australia, Washington	Shaw, 1973; Shivas, 1989; Farr and Rossman, 2018
	Prunus dulcis	California	Adaskaveg and Ogawa, 1990
	Prunus mahaleb	California	Adaskaveg and Ogawa, 1990
	Prunus mume	Japan	Kobayashi, 2007
	Prunus pensylvanica	Canada	Ginns, 1986
	Prunus persica	Australia, Bulgaria, California, Chile, China, Missouri, South Carolina, South Africa	Maneval, 1937; Shaw, 1973; Ginns, 1986; Cook and Dubé, 1989; Adaskaveg and Ogawa, 1990; Adaskaveg *et al.*, 1993; Crous *et al.*, 2000; Dai, 2005; Kobayashi, 2007; Farr and Rossman, 2018
	Prunus persica var. *vulgaris*	Japan	Kobayashi, 2007
	Prunus salicina	South Africa, Zimbabwe	Whiteside, 1996; Crous *et al.*, 2000
	Prunus serotina	Canada	Ginns, 1986

* Current name given, disease name given in brackets if known.

Table 2. Continue.

Causal organism*	Host	Country	Reference
	Prunus sp.	Georgia, Japan, Michigan, Oklahoma, Oregon, Washington	Preston, 1945; Campbell *et al.*, 1950; Shaw, 1973; Kobayashi, 2007; Farr and Rossman, 2018
	Prunus x *avium-cerasus*	South Africa	Gorter, 1977
	Prunus x *yedoensis*	Japan	Kobayashi, 2007
Schizopora paradoxa (Schrad.) Donk (White rot)	*Prunus emarginata*	Canada	Ginns, 1986
	Prunus persica	South Carolina	Adaskaveg *et al.*, 1993
	Prunus serrulata	South Korea	Lee *et al.*, 2002
	Prunus sp.	Russia	Farr & Rossman, 2018
Stereum hirsutum (Willd.) Pers. (White rot)	*Prunus avium*	Bulgaria	Farr & Rossman, 2018
	Prunus cerasus	Bulgaria, New York, Virginia	Chamuris, 1988; Farr and Rossman, 2018
	Prunus domestica	Bulgaria, Oregon	Shaw, 1973; Farr and Rossman, 2018
	Prunus dulcis	California	Adaskaveg and Ogawa, 1990
	Prunus pensylvanica	Canada	Ginns, 1986
	Prunus persica	Australia, Oregon, South Carolina	Shaw, 1973; Shivas, 1989; Adaskaveg *et al.*, 1993
	Prunus serotina	Massachusetts	Chamuris, 1988
	Prunus sp.	Canada, Japan, New York, Oregon, Vermont	Ginns, 1986; Chamuris, 1988; Kobayashi, 2007
Trichaptum biforme (Fr.) Ryvarden (White rot)	*Prunus mume*	Japan	Kobayashi, 2007
	Prunus persica	South Carolina	Adaskaveg *et al.*, 1993
	Prunus sp.	Japan	Kobayashi, 2007

* Current name given, disease name given in brackets if known.

Table 2. Continue.

Causal organism*	Host	Country	Reference
Trametes hirsuta (Wulfen) Lloyd (Heart rot; White rot)	*Prunus armeniaca*	California, New Zealand	Cunningham, 1965; Adaskaveg and Ogawa, 1990
	Prunus avium	Bulgaria	Farr and Rossman, 2018
	Prunus cerasus	Bulgaria	Farr and Rossman, 2018
	Prunus domestica	Bulgaria, New Zealand	Cunningham, 1965; Farr and Rossman, 2018
	Prunus maximowiczii	Japan	Kobayashi, 2007
	Prunus persica	California, New Zealand, South Carolina	Cunningham, 1965; Adaskaveg and Ogawa, 1990; Adaskaveg *et al.*, 1993
	Prunus sp.	California, China, Japan, Russia, Slovakia	Adaskaveg and Ogawa, 1990; Kobayashi, 2007; Farr and Rossman, 2018
	Prunus ssiori	Japan	Kobayashi, 2007
Trametes versicolor (L.) Lloyd (White rot)	*Prunus americana*	California, New Zealand	Adaskaveg and Ogawa, 1990; Gadgil, 2005
	Prunus armeniaca	Australia, California, Japan, New Zealand	Cunningham, 1965; Sampson and Walker, 1982; Adaskaveg and Ogawa, 1990; Kobayashi, 2007
	Prunus avium	Australia, California, New Zealand	Cunningham, 1965; Sampson and Walker, 1982; Adaskaveg and Ogawa, 1990
	Prunus cerasus	New Zealand	Cunningham, 1965
	Prunus domestica	California	Adaskaveg and Ogawa, 1990
	Prunus mahaleb	California	Adaskaveg and Ogawa, 1990
	Prunus mume	Japan	Kobayashi, 2007

* Current name given, disease name given in brackets if known.

Table 2. Continue.

Causal organism*	Host	Country	Reference
	Prunus persica	California, New Zealand, South Carolina; South Africa	Cunningham, 1965; Adaskaveg and Ogawa, 1990; Adaskaveg *et al.*, 1993; Crous *et al.*, 2000; Kobayashi, 2007
	Prunus salicina	Japan, New Zealand	Cunningham, 1965; Kobayashi, 2007
	Prunus sargentii	Japan	Kobayashi, 2007
	Prunus serrulata	South Korea	Lee *et al.*, 2002
	Prunus sp.	Australia, China, Japan, New Hampshire, Washington	Sampson and Walker, 1982; Cox *et al.*, 2006; Kobayashi, 2007; Farr and Rossman, 2018
	Prunus x *yedoensis*	Japan	Kobayashi, 2007

* Current name given, disease name given in brackets if known.

ADDENDUM B

Table 1. Analysis of variance for the effect of the different cultivar combinations from the nurseries on the trees infected with canker and wood rot pathogens for each combination.

Source	DF	F-Value	Pr > F
Nursery	2	0.12	0.8872
ScionRootstockCombination	8	1.32	0.3437

Table 2. Analysis of variance for the effect of the different cultivar combinations from the nurseries on the trees infected with "*Cylindrocarpon*"-like fungi for each combination.

Source	DF	F-Value	Pr > F
Nursery	2	3.54	0.0735
ScionRootstockCombination	8	5.70	0.0087

Table 3. Analysis of variance for the effect of the different plant parts isolated from the different cultivar combinations from the nurseries on the infection with canker and wood rot pathogens.

Source	DF	F-Value	Pr > F
Nursery	2	0.17	0.8409
ScionRootstockCombination	8	3.02	0.0190
ScionRootstockCombination(Nursery)	9	2.57	0.0344
PlantPart	2	3.22	0.0596
ScionRootstockCombinationxPlantPart	16	0.98	0.5114

Table 4. Analysis of variance for the effect of the different plant parts isolated from the different cultivar combinations from the nurseries on the infection with "*Cylindrocarpon*"-like fungi.

Source	DF	F-Value	Pr > F
Nursery	2	5.34	0.0129
ScionRootstockCombination	8	9.10	<.0001
ScionRootstockCombination(Nursery)	9	1.52	0.2007
PlantPart	2	84.71	<.0001
ScionRootstockCombinationxPlantPart	16	8.16	<.0001

Table 5. Analysis of variance for the effect of the infection by "*Cylindrocarpon*"-like fungi in the crown on the different cultivar combinations from the nurseries.

Source	DF	F-Value	Pr > F
Nursery	2	3.95	0.0586
ScionRootstockCombination	8	6.43	0.0058

Table 6. Analysis of variance for the effect of the different plant parts isolated from the different cultivar combinations from the nurseries on the infection with canker and wood rot pathogens.

Source	DF	F-Value	Pr > F
ScionRootstockCombination	8	2.68	0.0130
ScionRootstockCombination(Nursery)	9	1.90	0.0679
PlantPart	2	1.91	0.1559
ScionRootstockCombinationxPlantPart	16	0.87	0.6072
ScionRootstockCombination(Nursery)	22	0.81	0.7016
TaxonomicGroup	2	1.20	0.3078
ScionRootstockCombinationxTaxonomicGroup	16	2.67	0.0027
PlantPartxTaxonomicGroup	4	4.57	0.0026
ScionRootstockCombinationxPlantPart	32	1.22	0.2433

Table 7. Analysis of variance between isolates on Orchard 1 (African Rose) after 4 months of inoculation.

Source	DF	F-Value	Pr > F
Tree	153	3.47	< .0001
Isolate	66	13.69	< .0001

Table 8. Analysis of variance between isolates on Orchard 2 (Sunkiss) after 4 months of inoculation.

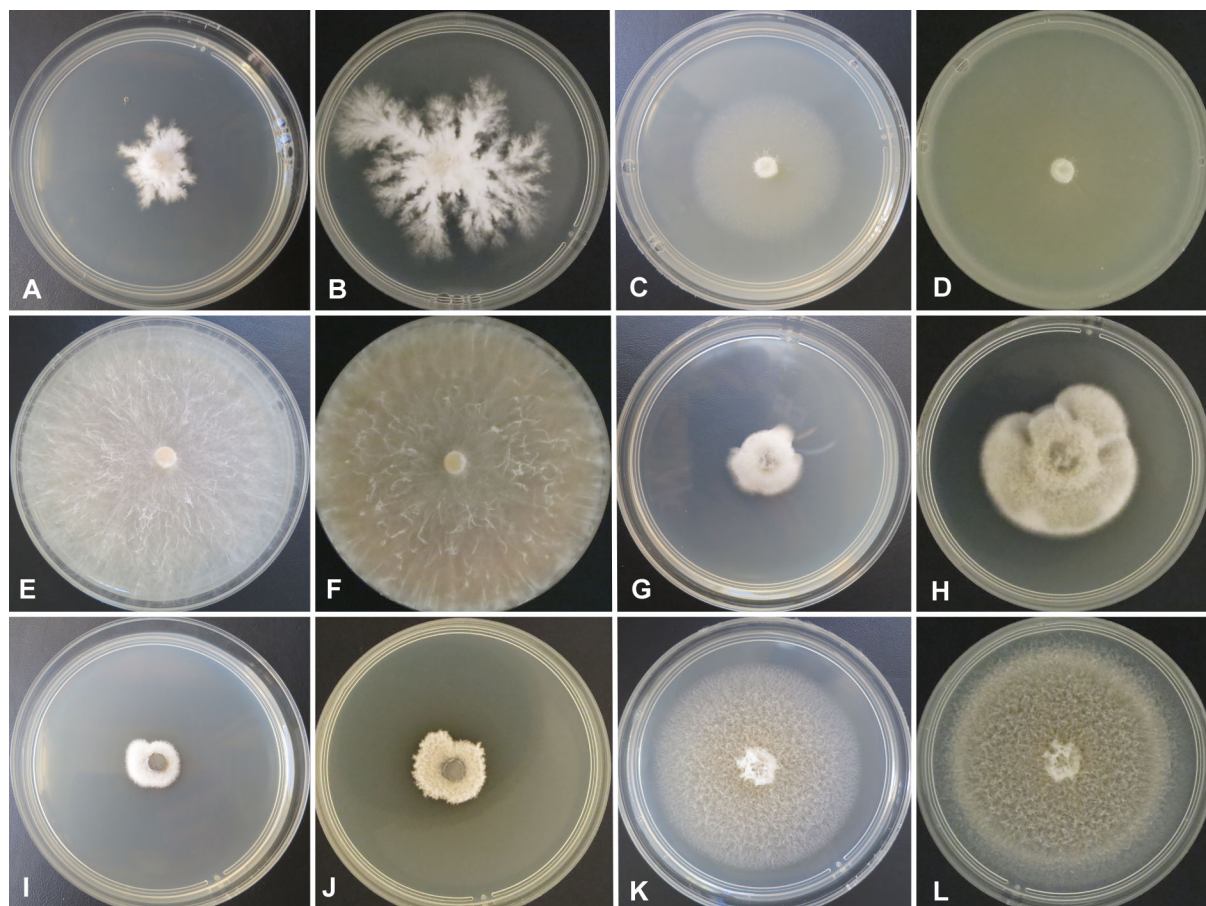| Source	DF	F-Value	Pr > F
Tree	153	2.29	< .0001
Isolate	66	7.13	< .0001

ADDENDUM C

Figure 1. Cultural growth of *Schizophyllum commune*, species within the Diatrypaceae, *Paraphoma chrysanthemicola*, *Pleurostoma richardsiae* and *Truncatella angustata* on PDA incubated at 25°C. *Schizophyllum commune* (STEU 8841) after 7 days (A) and 14 days (B); *Eutypella* sp. (STEU 8906) after 7 days (C) and 14 days (D); *Eutypa leptoplaca* (STEU 8905) after 7 days (E) and 14 days (F); *Paraphoma chrysanthemicola* (STEU 8924) after 7 days (G) and 14 days (H); *Pleurostoma richardsiae* (STEU 8936) after 7 days (I) and 14 days (J); *Truncatella angustata* (STEU 8835) after 7 days (K) and 14 days (L).
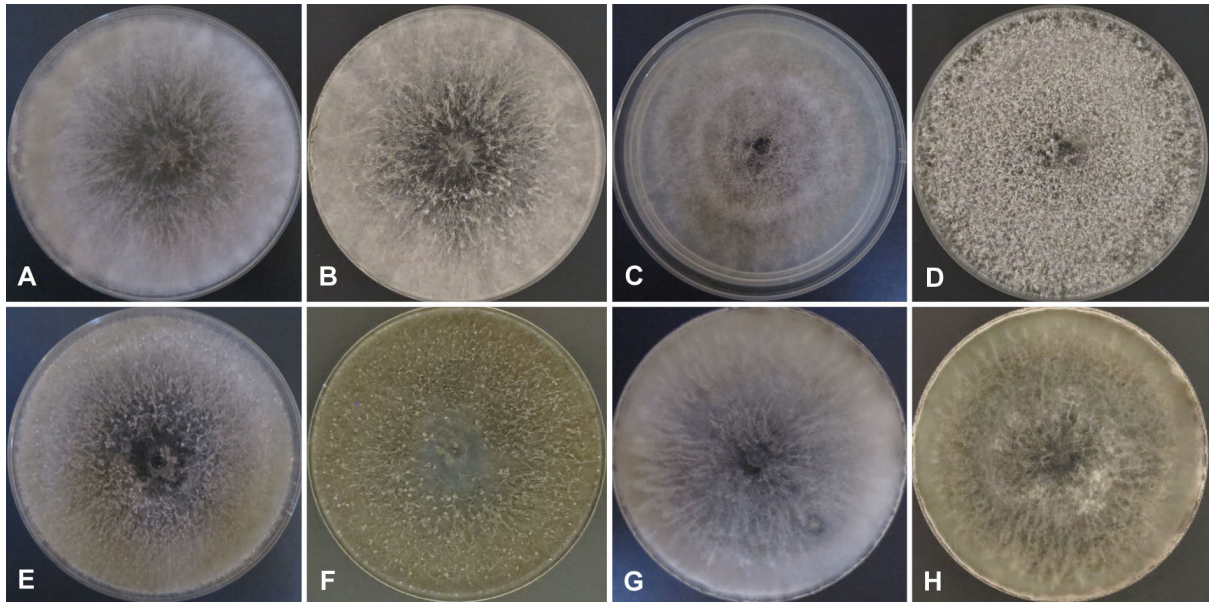
Figure 2. Cultural growth of species within Botryosphaeriaceae on PDA incubated at 25°C. *Diplodia seriata* (STEU 8845) after 7 days (A) and 14 days (B); *Dothiorella moneti* (STEU 8847) after 7 days (C) and 14 days (D); *Dothiorella viticola* (STEU 8848) after 7 days (E) and 14 days (F); *Lasiodiplodia theobromae* (STEU 8849) after 7 days (G) and 14 days (H).
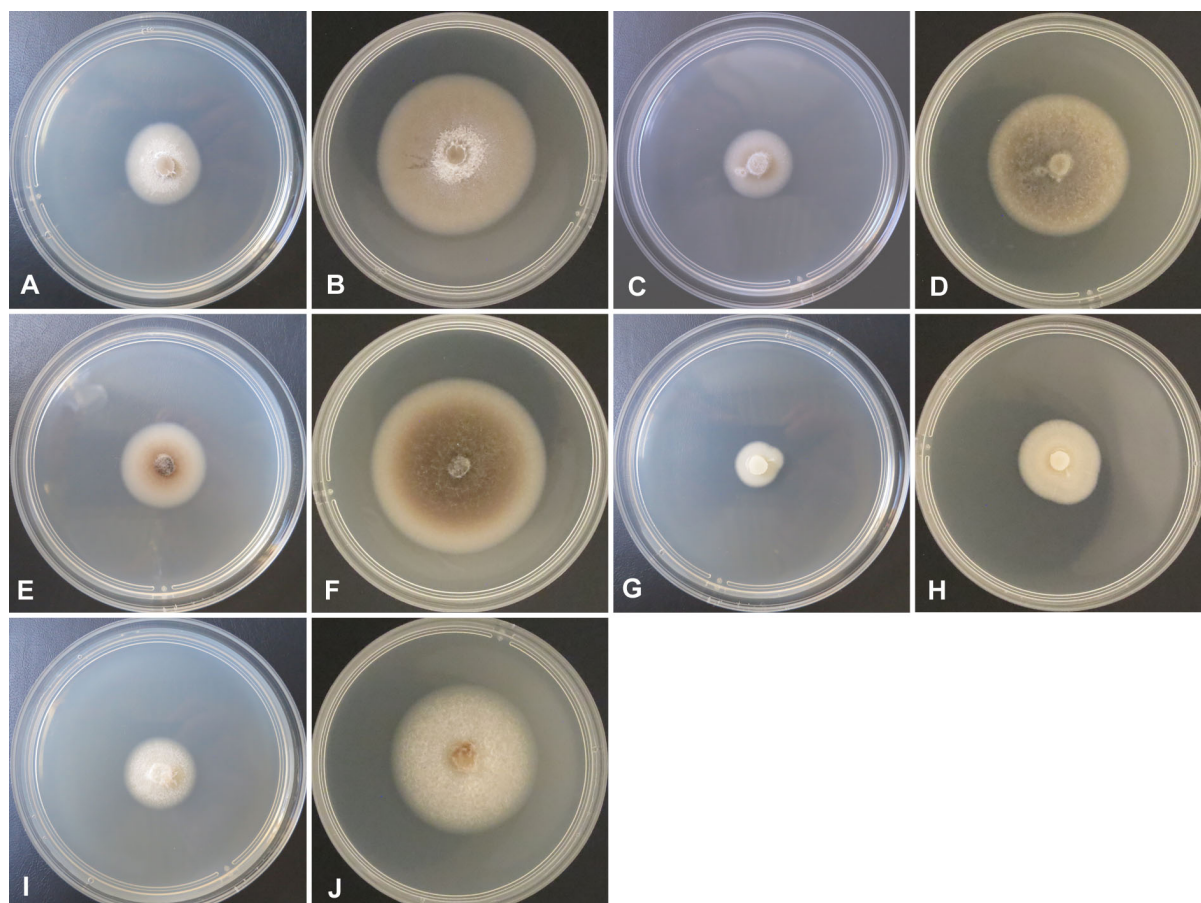
Figure 3. Cultural growth of *Cadophora* species on PDA incubated at 25°C. *Cadophora gregata* (STEU 8855) after 7 days (A) and 14 days (B); *Cadophora luteo-olivacea* (STEU 8856) after 7 days (C) and 14 days (D); *Cadophora malorum* (STEU 8858) after 7 days (E) and 14 days (F); *Cadophora novi-eboraci* (STEU 8860) after 7 days (G) and 14 days (H); *Cadophora* sp. 2 (STEU 8865) after 7 days (I) and 14 days (J).
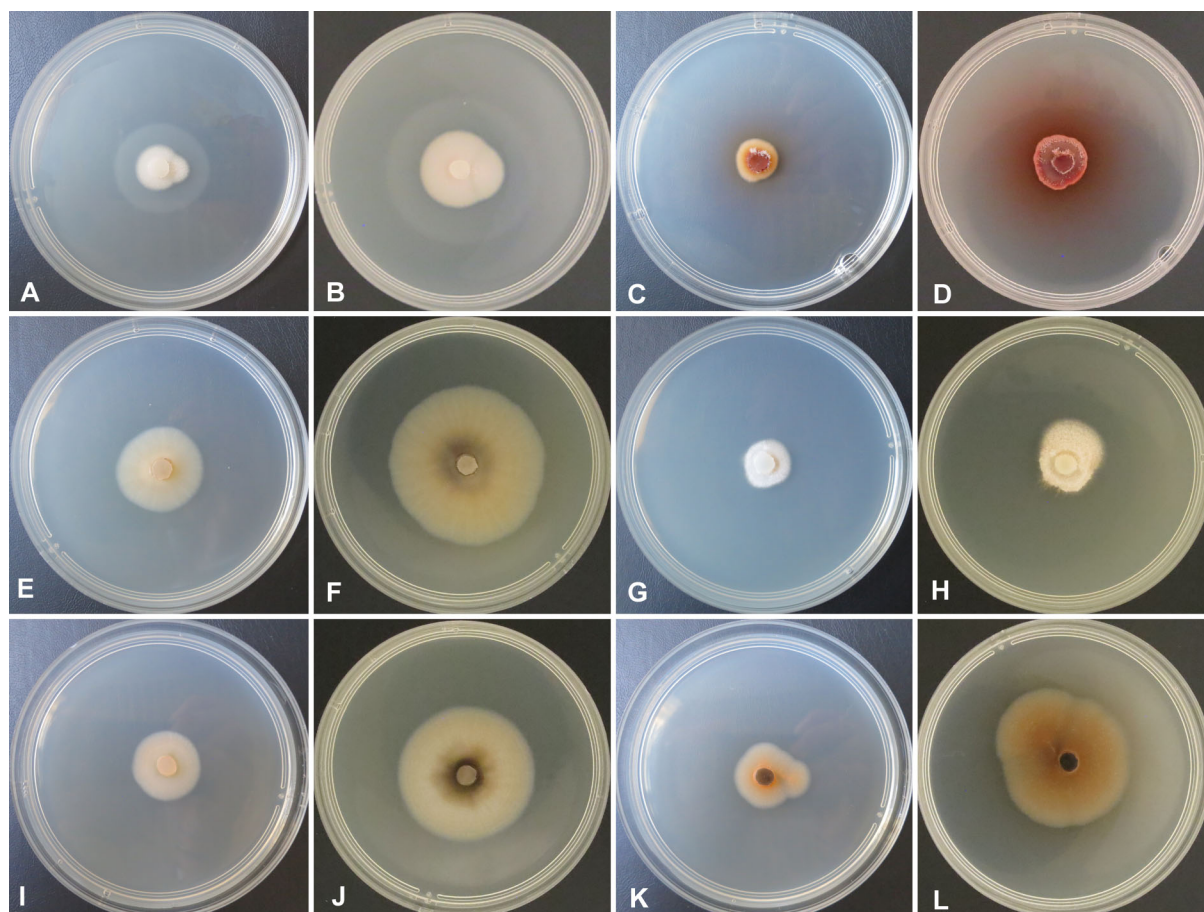
Figure 4. Cultural growth of *Collophorina* and *Coniochaeta* species on PDA incubated at 25°C. *Collophorina paarla* (STEU 8866) after 7 days (A) and 14 days (B); *Collophorina rubra* (STEU 8868) after 7 days (C) and 14 days (D); *Coniochaeta hoffmannii* (STEU 8869) after 7 days (E) and 14 days (F); *Coniochaeta* sp. 1 (STEU 8873) after 7 days (G) and 14 days (H); *Coniochaeta* sp. 2 (STEU 8876) after 7 days (I) and 14 days (J); *Coniochaeta velutina* (STEU 8879) after 7 days (K) and 14 days (L).
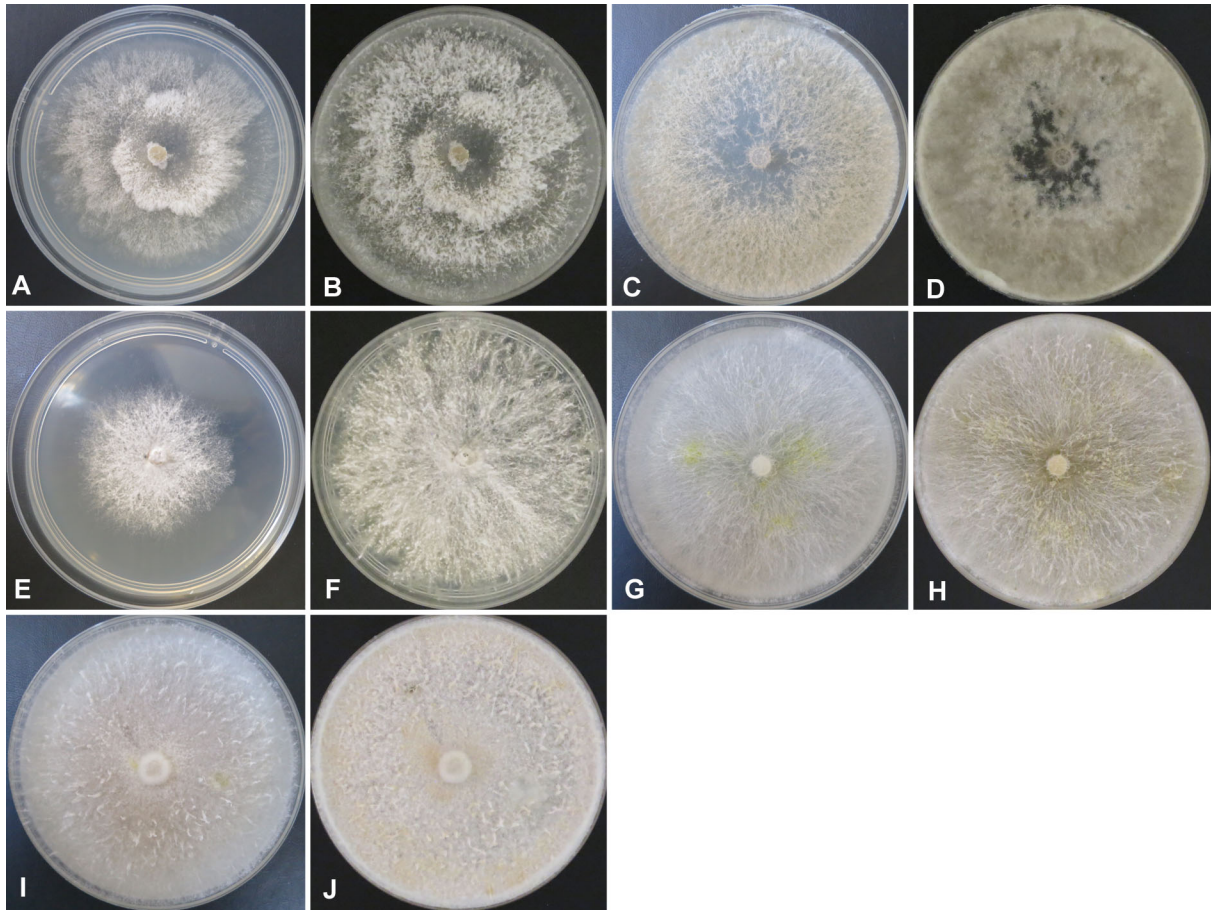
Figure 5. Cultural growth of species within Diaporthaceae and *Biscogniauxia* species on PDA incubated at 25°C. *Diaporthe ambigua* (STEU 8902) after 7 days (A) and 14 days (B); *Diaporthe aspalathi* (STEU 8903) after 7 days (C) and 14 days (D); *Diaporthe foeniculina* (STEU 8904) after 7 days (E) and 14 days (F); *Biscogniauxia mediterranea* (STEU 8842) after 7 days (G) and 14 days (H); *Biscogniauxia* sp. (STEU 8843) after 7 days (I) and 14 days (J).
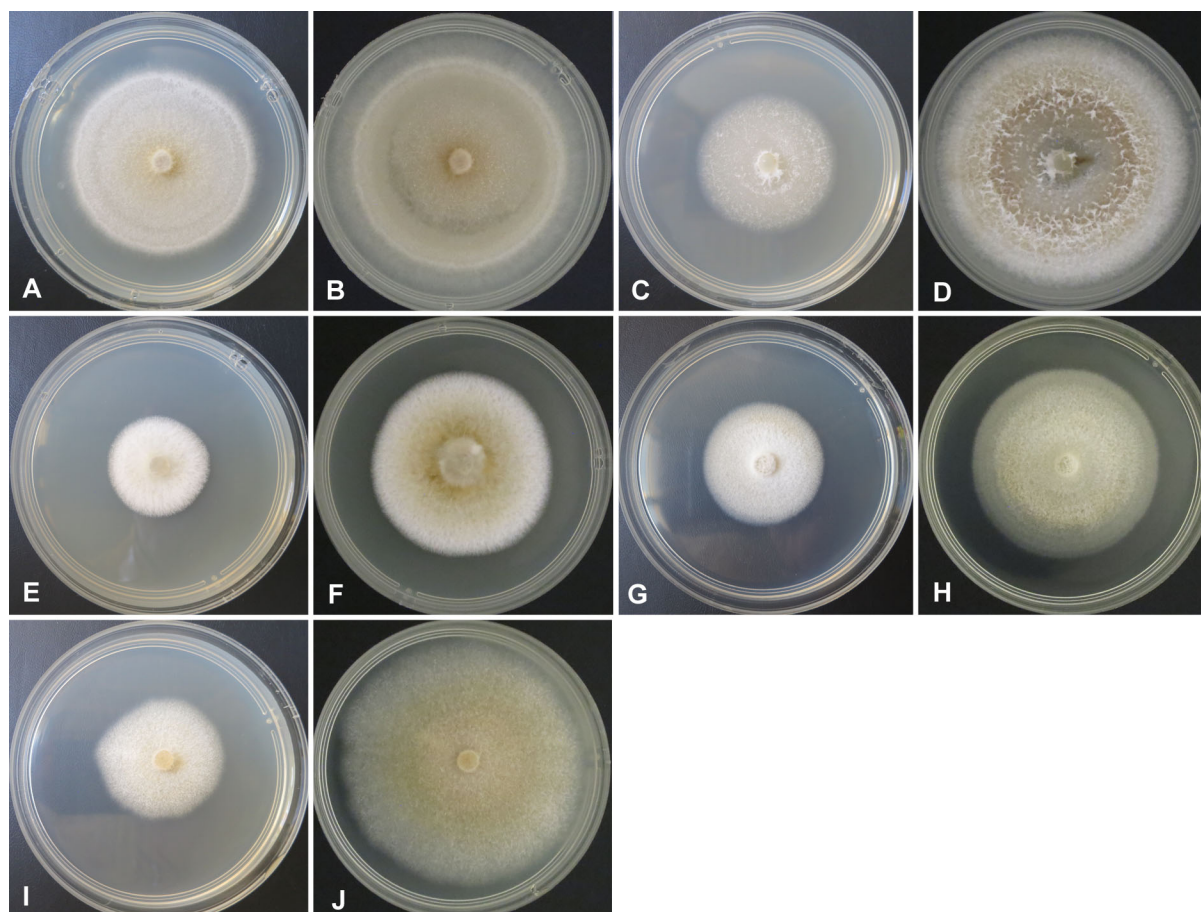
Figure 6. Cultural growth of *species within* Didymellaceae and Didymosphaeriaceae on PDA incubated at 25°C. *Didymella pomorum* (STEU 8908) after 7 days (A) and 14 days (B); *Didymosphaeria rubi-ulmifolii* (STEU 8911) after 7 days (C) and 14 days (D); *Didymosphaeria variabile* (STEU 8913) after 7 days (E) and 14 days (F); *Paraphaesphaeria neglecta* (STEU 8921) after 7 days (G) and 14 days (H); *Paraphaeosphaeria sporulosa* (STEU 8922) after 7 days (I) and 14 days (J).
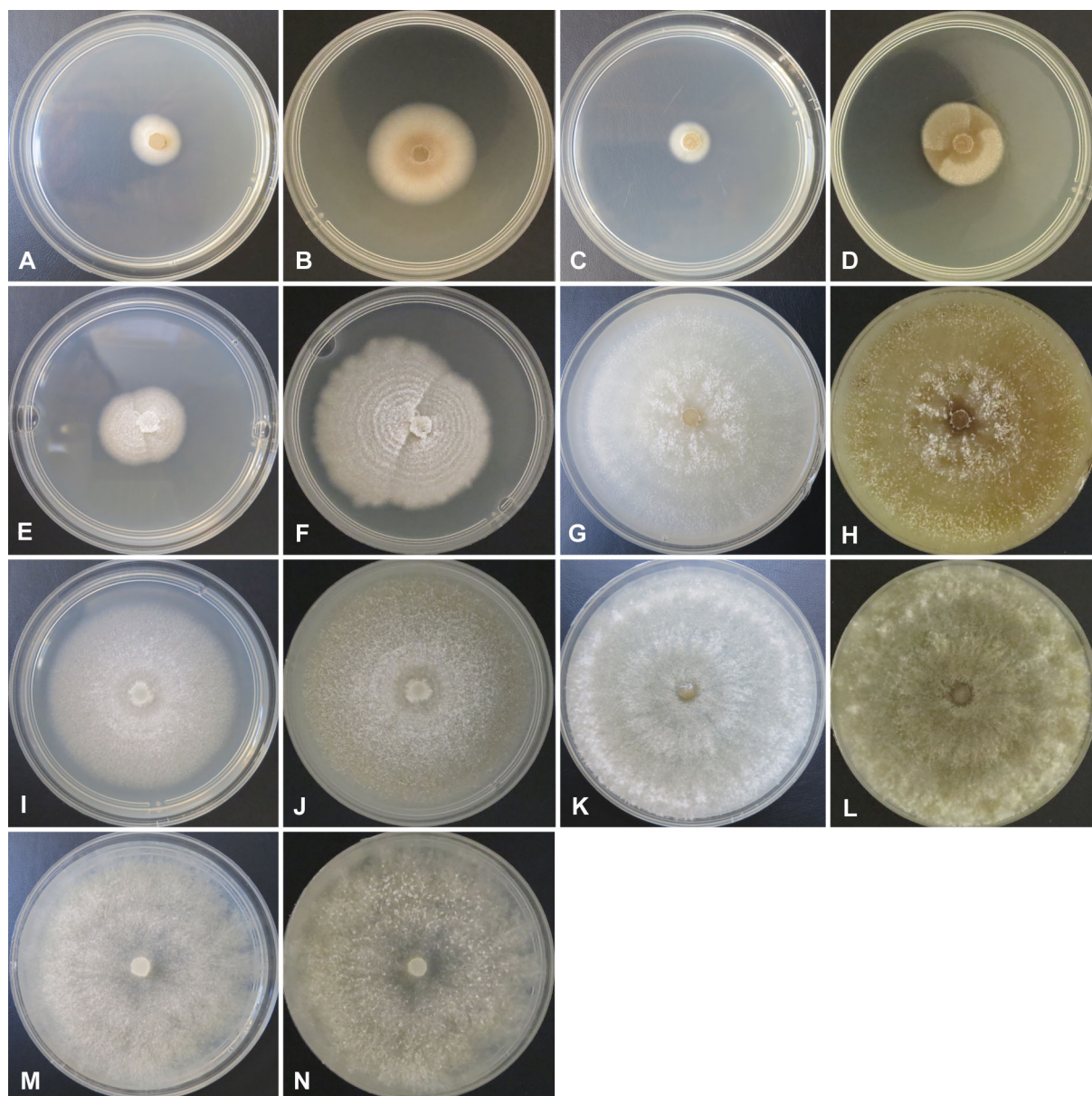
Figure 7. Cultural growth of *Phaeoacremonium* species and species within Valsaceae on PDA incubated at 25°C. *Phaeoacremonium parasiticum* (STEU 8934) after 7 days (A) and 14 days (B); *Phaeoacremonium iranianum* (STEU 8929) after 7 days (C) and 14 days (D); *Cytospora austromontana* (STEU 8880) after 7 days (E) and 14 days (F); *Cytospora* sp. 1 (STEU 8883) after 7 days (G) and 14 days (H); *Cytospora* sp. 2 (STEU 8887) after 7 days (I) and 14 days (J); *Cytospora leucostoma* (STEU 8881) after 7 days (K) and 14 days (L); *Valsa sordida* (STEU 8888) after 7 days (M) and 14 days (N).