South African Journal of Botany xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Fusarium species associated with damping-off of rooibos seedlings and the potential of compost as soil amendment for disease suppression

S.C. Lamprecht *, Y.T. Tewoldemedhin

Agricultural Research Council, Plant Protection Research, Private Bag X5017, Stellenbosch 7599, Western Cape, South Africa

ARTICLE INFO

Available online xxxx

Edited by E Joubert

Keywords:
Rooibos
Fusarium oxysporum
Fusarium foetens
Lupin
Oat
Compost

ABSTRACT

Fusarium species associated with damping-off of rooibos in the Western Cape Province of South Africa were recovered from 12 nurseries during the 2007 to 2009 seasons. All 121 isolates obtained, morphologically resembled *Fusarium oxysporum*. Of the 58 representative isolates identified through sequence analyses of the EF-1α gene area, 25 were *F. foetens* and 33 *F. oxysporum*, and these two species were obtained from 11 and 12 of the nurseries, respectively. Two strongly supported clades (Clades 2 and 3) of the *Fusarium oxysporum* species complex were recovered in this study, while a well-supported clade corresponded to the clade reported as *F. foetens*. Twenty isolates of each *Fusarium* sp. were evaluated for their ability to cause damping-off of rooibos, and the rotation crops, lupin and oat, under glasshouse conditions. Both *Fusarium* spp. caused significant damping-off of rooibos seedlings, but not of lupin or oat. However, both species could be reisolated from lupin, but not from oat. This is the first report of these two *Fusarium* spp. as pathogens of rooibos seedlings, and also the first report of *F. foetens* in South Africa. Two commercial composts (compost A and compost B) from independent suppliers were evaluated for disease suppression under glasshouse conditions. Both composts significantly suppressed damping-off caused by both *Fusarium* spp., but in a few instances, compost B was more effective than compost A in disease suppression of individual isolates of both species.

1. Introduction

Rooibos (*Aspalathus linearis* (N.L. Burm.) R. Dahlgr.), an important indigenous herbaceous legume crop in South Africa, is only grown in the Cederberg mountainous region of the Western Cape. This perennial plant is used to produce herbal teas and also a few other products (Joubert et al., 2008). Rooibos seedlings are produced in open-bed nurseries. Seeds are planted in February to March, and 4 months after planting, seedlings are transplanted into open-field plantations. Rooibos seedlings in nurseries are very susceptible to damping-off and losses of 50% or more can occur if control measures are not applied. It is known that damping-off occurs within the first six weeks after planting (unpublished data) and that the disease is caused by a complex of pathogens, including *Fusarium*, *Pythium* and *Rhizoctonia* spp. (Bahramishariff et al., 2014).

Bahramishariff et al. (2014) identified several *Pythium* spp. associated with rooibos seedlings in nurseries viz. *Pythium acanthicum* Drechsler, *P. irregulare* Buisman, *P. mamillatum* Meurs, *P. myriotylum* Drechsler, *P. pyrilobum* Vaartaja, *P. cederbergense* Bahramisharif, Botha & Lamprecht and *Pythium* RB II, with *P. irregulare* being the most common species associated with damped-off seedlings. All the *Pythium* spp. except *P. acanthicum* Dreschler, *P. cederbergense* and *Pythium* RB II were pathogenic on rooibos seedlings and highly virulent. *Phytophthora (Ph.) cinnamomi* Rands isolated from rooibos collected from a native site was also shown to be highly virulent, but was not obtained from diseased seedlings in nurseries (Bahramishariff et al., 2014). In a more recent study, Tewoldemedhin et al. (2015) characterised the *Rhizoctonia* species and anastomosis groups (AGs) associated with diseased rooibos seedlings in nurseries. The multinucleate isolates included four AGs in *Rhizoctonia solani* Kühn, AG 2-2, AG-4 HGI and AG-11 as well as *R. zeae* Voorhees. The binucleate AGs obtained were AG-Bo and AG-K as well as an unidentified binucleate *Rhizoctonia*. The most virulent *Rhizoctonia* AGs on rooibos were AG-2-2, AG-4 HGI and AG-11. Although the specific *Pythium* spp. and *Rhizoctonia* spp. and AGs associated with damping-off of rooibos have been identified, there is, with the exception of a record of *F. chlamydosporum* Wollenweber & Reinking causing damping-off of rooibos seedlings (Engelbrecht et al., 1983), no information on *Fusarium* spp. responsible for damping-off of rooibos in nurseries.

Management of soilborne diseases often relies on the integration of different strategies. Two of these strategies include crop rotation and amendment of soil with compost (Noble and Coventry, 2005; Lamprecht et al., 2006). It is known that crop rotation with different nonhost crops differs in their ability to reduce losses caused by soilborne diseases (Lamprecht et al., 2006). Rooibos in nurseries is rotated with

* Corresponding author at: Agricultural Research Council, Plant Protection Research Institute, Private Bag X5017, Stellenbosch 7599, Western Cape, South Africa. Tel.: +27 21 887 4690; fax: +27 21 887 5096.
E-mail address: lamprechts@arc.agric.za (S.C. Lamprecht).

http://dx.doi.org/10.1016/j.sajb.2016.07.009
0254-6299/

lupin (*Lupinus angustifolius* L.) and oat (*Avena sativa* L.), and in the studies conducted by Bahramishariff et al. (2014) it was shown that the *Pythium* species highly virulent on rooibos are less virulent on lupin and oat. In addition, Tewoldemedhin et al. (2015) concluded that with regards to the susceptibility of lupin and oat to Rhizoctonia pathogens of rooibos, oat is a better precrop than lupin prior to the establishment of rooibos. However, the efficiency of these crops to reduce diseases of rooibos caused by *Fusarium* species is unknown.

Application of organic amendments, such as compost, has been reported as a management strategy against soilborne pathogens, including *Fusarium*, *Pythium* and *Rhizoctonia* spp. (Noble and Coventry, 2005). Bahramisharif et al. (2013) and Tewoldemedhin et al. (2015) evaluated suppression of *Pythium* and *Rhizoctonia* spp. with two composts and reported within and between species and AG variation. In order to determine the suitability of compost as a management strategy against damping-off in organic rooibos nurseries, it is important to also establish the effect of compost on suppression of *Fusarium* spp. associated with damping-off of rooibos seedlings.

The aims of our study were to determine (i) the *Fusarium* spp. associated with damping-off of rooibos seedlings in 12 nurseries, (ii) the pathogenicity of *Fusarium* spp. toward rooibos and two rotation crops (lupin and oat) and (iii) determine whether damping-off of rooibos caused by *Fusarium* spp. could be suppressed by compost under controlled conditions.

2. Materials and methods

2.1. Isolations

Rooibos seedlings with damping-off symptoms were collected from 12 rooibos nurseries in March and April 2007 to 2009. Roots and hypocotyls of symptomatic seedlings were washed and then surface disinfested in 1% sodium hypochlorite, rinsed twice in sterile distilled water, and allowed to dry in a laminar flow cabinet. Pieces of diseased root and hypocotyl tissue were plated onto the following growth media: water agar (WA), water agar with 0.02% novostreptomycin (WA+), and potato dextrose agar with 0.02% novostreptomycin (PDA+). Twenty four pieces of plant material (12 root and 12 crown) were plated per nursery. All fungi that developed from the plant material were transferred to carnation leaf agar plates (Fisher et al., 1982). All fungi with cultural characteristics typical of *Fusarium* spp. were purified by single-sporing (Nelson et al., 1983). Cultures were lypholised and also stored on potato-carrot agar slants in a cold room at 10 °C.

2.2. Molecular characterisation of Fusarium isolates

DNA was isolated from 5-day-old *Fusarium* cultures growing on PDA+ plates. Fungal mycelia were transferred to a 2.2 mL centrifuge tube containing 1 mL SDS extraction buffer and 0.5 g glass beads. Mycelia were lysed by shaking the tubes for 5 min in a mixer/miller (Retsch® MM301, GmbH & Co., Haan, Germany) at maximum speed. DNA was isolated from the lysed cells as described by Lee and Taylor (1990). DNA integrity was checked by running DNA samples on 1.0% agarose gels, followed by ethidium bromide staining. DNA was visualised and photographed under UV illumination. DNA quantification was conducted using a NanoDrop (NanoDrop Technologies, Wilmington, DE, USA).

The translocation elongation factor (EF-1α) gene was amplified from *Fusarium* isolates using primers EF-1 (5′-ATGGGTAAGGARGACAAGAC-3′) and EF-2 (5′-GGARGTACCAGTSATCATG-3′) (O'Donnell et al., 1998). Polymerase chain reaction (PCR) reactions using the primer pairs consisted of 0.2 μM of each forward and reverse primer, 200 μM of each dNTP, 1× PCR buffer (Bioline, Inc., Taunton, MA), 0.65 U BIOTAQ™ DNA polymerase (Bioline), 0.2 mg bovine serum albumin (BSA) Fraction V (Roche Diagnostics Randburg, South Africa), 2 μL of DNA (5–10 ng) and 2 mM MgCl2 in a final volume of 40 μL. Amplifications were conducted in a thermocycler (2700 PCR System, GeneAmp®, Applied Biosystems, Foster City, CA), starting with an initial denaturation of 5 min at 94 °C, followed by 32 cycles of 45 s at 94 °C, 45 s at 53 °C and 90 s at 72 °C and a final extension cycle of 7 min at 72 °C.

PCR products (5 μL aliquot) were electrophoresed through 1.0% agarose gels containing ethidium bromide in 1× Tris-acetate EDTA buffer. DNA fragments were visualised under UV illumination and samples that amplified were cleaned using the MSB® Spin PCRapace (Invitek, Berlin, Germany) kit according to manufacturer's instructions. PCR products were sequenced with primers EF-1 and EF-2. Sequencing reactions were performed in a total volume of 10 μL containing 2 μL of DNA (10 to 20 ng), 0.4 μM primer, 1 μL BigDye system master mix, and 2 μL of 5× sequencing buffer (Applied Biosystems). Sequence amplification condition consisted of an initial denaturation at 94 °C for 5 min followed by 25 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s, with a final extension step at 72 °C for 7 min. Sequencing reactions were run on a ABI 3130XL Genetic Analyzer (Applied Biosystems). Geneious Pro 3.6.2 (Biomatters Ltd., Auckland, New Zealand) was used to view ABI trace files.

Sequences obtained from *Fusarium* isolates were submitted to BLAST analyses in the FUSARIUM-ID v. 1.0 database (http://fusarium.cbio.psu.edu) (Geiser et al., 2004).

Phylogenetic analyses of the EF-1α gene sequence data. The *Fusarium* sequences (n = 30) were aligned in Geneious Pro 3.6.2. Reference sequences (NRRL isolates of Kerry O′Donnell) were also included in the phylogenetic analysis. *Fusarium* sp. (NRRL25184) sequence was used as an out-group.

Sequences were aligned online using the software MAFFT alignment program version 6 (Katoh and Toh, 2008). Alignments were adjusted manually using the software Geneious Pro 3.6.2. Maximum parsimony and distance analysis were conducted in PAUP (Phylogenetic Analysis Using Parsimony) 4.0b10 (Swofford, 2002). For distance analysis, neighbour-joining with the uncorrected "p" model was performed. Maximum parsimony analysis was performed using the heuristic search option with a 100 random taxon additions. Tree bisection and reconstruction (TBR) was used as the branch swapping algorithm with the option of saving no more than 10 trees with a score greater than or equal to 5 (Harrison and Langdale, 2006). Gaps were treated as missing data. All characters were unordered and of equal weight. Bootstrap support values were calculated from 1000 heuristic search replicates and 100 random taxon additions. Other measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and the rescaled consistency index (RC) values.

2.3. Pathogenicity and cross-pathogenicity bioassays

Forty representative *Fusarium* isolates recovered from rooibos were evaluated for pathogenicity on rooibos as well as the potential rotation crops lupin and oat. The selected isolates included *Fusarium foetens* Schroers, O′Donnell, Baayen & Hooftman (20 isolates) and *F. oxysporum* Schlecht. emend. Snyder & Hansen (20 isolates). The pathogenicity trial was conducted in a glasshouse (day and night temperatures of 28 and 18 °C). The planting medium consisted of a pasteurised soil, perlite and sand mix (equal amounts) inoculated with a sand-bran inoculum of each isolate 3 days after pasteurisation of the planting medium. The sand-bran inoculum was prepared according to Lamprecht (1986) and was mixed with the planting medium at a concentration of 0.05% (wt/wt). Controls consisted of uninoculated sand-bran. Plastic pots (13 cm diameter) were filled with 800 g of the inoculated media. Ten planting holes were made in each pot to a depth of 1.5 cm (rooibos) and 2 cm (lupin and oat). Seeds that were planted one day after inoculum was mixed with the planting medium and pots were watered every alternate day. The number of seed planted per pot was 50 for rooibos and oat, and 20 for lupin. There were three replicates for each treatment in a completely randomised block design and the trial was conducted twice. Pathogenicity and virulence of the

Please cite this article as: Lamprecht, S.C., Tewoldemedhin, Y.T., *Fusarium* species associated with damping-off of rooibos seedlings and the potential of compost as soil amendment for disease suppression, South African Journal of Botany (2016), http://dx.doi.org/10.1016/j.sajb.2016.07.009