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Inoculum sources of *Fusarium oxysporum* f.sp. *cepae* on onion in the Western Cape Province of South Africa

Michael J. Southwood, Altus Viljoen, Adéle McLeod*

Department of Plant Pathology, Stellenbosch University, Private Bag X1, Matieland, 7600, South Africa

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ABSTRACT

Fusarium basal rot of onion, caused by *Fusarium oxysporum* f.sp. *cepae*, is one of the leading causes of post-harvest and storage losses within the Western Cape region in South Africa. Several vegetative compatibility groups (VCGs) of *F. oxysporum* f.sp. *cepae* have been associated with bulb rot in mature onions in South Africa, of which VCG 0425 predominates. Our study investigated seed and seedling transplants as potential sources of inoculum of *F. oxysporum* f.sp. *cepae,* and whether VCG 0425 is associated with these materials. *Fusarium* isolation studies from 13 seed lots showed that seven of the seed lots were infected with either moderately or highly virulent *F. oxysporum* f.sp. *cepae* isolates. The infection frequency of seed lots was between 0.17 and 0.50%, and only two of the seed lots were infected with VCG 0425. The seedborne nature of *F. oxysporum* f.sp. *cepae* was confirmed by showing that a green fluorescent protein (GFP)-labelled *F. oxysporum* f.sp. *cepae* VCG 0425 transformant could be transmitted from infected soil to bulbs, and from there to the seed stalks and seeds. Onion seedling transplants from nurseries were also implicated as a source of inoculum. The incidence of *F. oxysporum* f.sp. *cepae* in nurseries increased as the season progressed from 2.7% at the 6-week-old growth stage to 5.7% at the 14-week-old stage. The *F. oxysporum* f.sp. *cepae* isolates from transplants were highly to moderately virulent. However, none of the isolates proved to be VCG 0425. Most (>86%) of the *F. oxysporum* isolates from seed and seedlings were not *F. oxysporum* f.sp. *cepae* and were not pathogenic to onion. Altogether, the results indicate that onion seed and seedlings are inoculum sources of *F. oxysporum* f.sp. *cepae* in the Western Cape Province, but that VCG 0425 is rarely associated with these sources.

1. Introduction

Fusarium basal rot of onion (*Allium cepa*), caused by *Fusarium oxysporum* Schlechtend.:Fr. f.sp. *cepae* (H.N. Hans.) W.C. Snyder & H.N. Hans., has been identified as one of the leading causes of harvest and post-harvest storage losses of intermediate day-length onion types in the Western Cape region in South Africa (Southwood et al., 2012a). Onion bulb producers in the region establish their crops through seedling transplants obtained from nurseries, since direct seeding is not a successful crop production practice. Losses are particularly severe when hot and dry conditions prevail during spring, the time at which bulb initiation and active bulb growth occur (R.A. du Toit, Coordinating Committee for Onion and Potato, South Africa, personal communication).

Fusarium basal rot can cause losses from the seedling stage through to the post-harvest storage stage. *F. oxysporum* f.sp. *cepae* can infect onion seedlings, causing damping-off or delayed emergence. Older plants and mature bulbs can also start showing symptoms in the field, which include basal plate necrosis and rot of the inner basal scales and sometimes curving and yellowing of leaves and wilting at any plant growth stage. Most severe losses are encountered during post-harvest storage, where infected bulbs develop a dry rot of the basal plate and surrounding area, which sometimes develops into a soft rot due to secondary bacterial infections (Cramer, 2000; Crowe, 2008; Dissanayake et al., 2009b; Swift et al., 2002).

Soilborne *F. oxysporum* f.sp. *cepae* inoculum sources, mainly chlamydospores, can germinate and infect onion seedlings and bulbs through direct penetration of the basal plate. Infection can also occur through natural wounds in the basal plate and through roots, or through onion tissues that were damaged by maggot, smut or pink root. When soil inoculum levels are high, infection can also occur through basal portions of bulb scales (Cramer, 2000; Crowe, 2008; Everts et al., 1985; Koike et al., 2007; Sherf and Macnab,

* Corresponding author.
E-mail address: adelem@sun.ac.za (A. McLeod).

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1986). Following infection, symptom development and expression are influenced by environmental conditions (Crowe, 2008).

Onion seed and seedlings have been implicated as primary inoculum sources of *F. oxysporum* f.sp. *cepae*. It is well-known that *F. oxysporum* f.sp. *cepae* can successfully disseminate via infected onion seedling transplants over long distances, causing Fusarium basal rot symptoms on mature bulbs (Abawi and Lorbeer, 1972; Crowe, 2008; Özer and Köycü, 2004). However, the contribution of seed as an inoculum source is uncertain, since reports on the seedborne nature of *F. oxysporum* f.sp. *cepae* have only been published in non-peer reviewed journals that are not readily available, or that are not in English (references within Özer and Köycü, 2004). Although Köycü and Özer (1997) isolated *F. oxysporum* from onion seed embryos and reported that it was seedborne, they did not determine the pathogenicity of the isolates. In other crops including basil, tomato and lettuce, the seedborne nature of some *formae speciales* of *F. oxysporum* has been shown (Chiocchetti et al., 2001; Gamliel et al., 1996; Garibaldi et al., 2004; Menzies and Jarvis, 1994; Pasquali et al., 2005, 2006, 2007).

F. oxysporum f.sp. *cepae* is known as a genetically diverse pathogen of onions that can be characterized through gene phylogenies, DNA fingerprinting and vegetative compatibility groups (VCGs). Although VCG typing is conducted on a phenotypical basis, it provides an indication of the genetic relatedness of isolates. Most studies that have investigated the genetic diversity in *F. oxysporum* f.sp. *cepae,* did not clearly specify the onion developmental stage from which the pathogen was isolated (Dissanayake et al., 2009a, b; Galván et al., 2008; Southwood et al., 2012 a, b; Swift et al., 2002). Consequently, it is not clear whether the same *F. oxysporum* f.sp. *cepae* genotypes attack onion seedlings, mature plants and mature onion bulbs in storage. In South Africa, it has clearly been shown that mature harvested onion bulbs are predominantly infected by VCG 0425. Eighty nine percent of 92 isolates from mature bulbs collected from 2005 to 2007 were shown to be VCG 0425. The remaining isolates belonged to a few other VCGs (VCG 0423, 0426 and 0427) and single member VCGs (SMVs 1, 2, 5 and 6) (Southwood et al., 2012 a, b). VCG 0425 can be identified using sequence-characterized amplified region (SCAR) markers and random amplified polymorphic DNA (RAPD) fingerprinting (Southwood et al., 2012b).

Several studies have used fluorescent protein labelled *F. oxysporum formae speciales* isolates to learn more about their ecology and infection strategies. Important aspects of the colonization of host roots by several different *forma speciales* including *F. oxysporum* f.sp. *radicis-lycopersici, F. oxysporum* f.sp. *cubense, F. oxysporum* f.sp. *fragariae* and *F. oxysporum* f.sp. *niveum* were revealed for the first time using green fluorescent protein (GFP) labelled isolates (Laqopodi et al., 2002; Li et al., 2011; Lu et al., 2014; Yuan et al., 2014). Different coloured autofluorecent protein labelled isolates can also be used to investigate the interactions between biocontrol *F. oxysporum* strains and *F. oxysporum forma speciales* (Bolwerk et al., 2005). Fluorescently labelled *F. oxysporum* isolates have not been used to study seed infection on any crop, or infection of onion by *F. oxysporum* f.sp. *cepae.*

Due to the persistence of *F. oxysporum* f.sp. *cepae* in soil (Flood, 2006; Freeman et al., 2002), it is important that (i) the pathogen must not be introduced into pathogen-free soil or (ii) inoculum levels should not be increased in already infested soil through the introduction of external inoculum. The latter is important since a direct correlation has been found between *F. oxysporum* f.sp. *cepae* inoculum density and damping-off of onion seedlings (Abawi and Lorbeer, 1972). Therefore, the main aim of the present study was to determine whether onion seed and seedlings are potential inoculum sources of *F. oxysporum* f.sp. *cepae* in the Western Cape region of South Africa. This was done by means of isolation and pathogenicity studies of *F. oxysporum* isolates obtained from onion seeds and seedling transplants, and genotyping the isolates with VCG 0425 SCAR markers and RAPD fingerprinting (Southwood et al., 2012b). The seedborne nature of *F. oxysporum* f.sp. *cepae* was also investigated by employing a GFP reporter gene-labelled *F. oxysporum* f.sp. *cepae* isolate in artificial inoculation studies under laboratory conditions.

2. Materials and methods

2.1. Analysis of onion seed for the presence of Fusarium oxysporum and F. oxysporum f.sp. cepae

Onion seeds from two semi-commercial (HT49 and Cream Gold) and 11 commercial onion varieties were analysed. In total, the varieties included three local and three imported open-pollinated varieties, as well as seven *Filial 1* (F1) hybrid varieties (Table 1). Seeds were obtained from various local and internationally based vegetable seed companies.

For each variety, a total of 600 seeds were analysed. Half of the seeds were rinsed for 30 s in sterile distilled water, surface sterilized for 60 s in 70% alcohol and dried in a laminar-flow cabinet. The other half was not surface sterilized, but was plated out with their original commercial fungicide treatment intact. Seeds were plated onto Petri dishes (90 mm in diameter) containing potato dextrose agar (PDA; Biolab, Gauteng, South Africa) supplemented with 0.04 g streptomycin sulphate per litre agar (PDA^{+}), 10 seeds per Petri dish.

The Petri dishes were incubated at 22 °C ± 4 °C on the laboratory bench, and were inspected on a weekly basis for fungal growth. Where conidial morphology was typical of *F. oxysporum*, the *Fusarium* colonies were purified using hyphal tipping and single-sporing (Leslie and Summerell, 2006). Single-spored isolates were stored in 15% glycerol at −80 °C.

2.2. Identification and characterization of F. oxysporum f.sp. cepae isolates

The pathogenicity of *F. oxysporum* isolates from onion seed and seedling transplants (see point 2.3) was evaluated using a previously described onion bulb inoculation method (Southwood et al., 2012a). Pathogenicity testing of each isolate was conducted using four onion bulbs of the onion variety Coastal Cream. Each pathogenicity assay included negative controls consisting of a non-pathogenic *F. oxysporum* isolate and a water control, as well as a positive control consisting of a highly virulent *F. oxysporum f.sp. cepae* isolate. Isolates were identified as *F. oxysporum f.sp. cepae* if they caused between 20 and 100% necrosis of the cut basal plate. The *F. oxysporum f.sp. cepae* isolates were further sub-divided into highly virulent (70–100% necrosis) and moderately virulent (20–69% necrosis) isolates. Isolations were made from bulbs that developed more than 20% necrosis, to fulfil Koch's postulates. Isolates causing no or less than 10% necrosis of the cut basal plate section were considered non-pathogenic (Southwood et al., 2012a). Each trial was conducted twice.

The presence of VCG 0425 isolates among the *F. oxysporum* f.sp. *cepae* isolates was investigated using a previously developed inter-retrotransposon sequence characterized amplified region (IR-SCAR) multiplex polymerase chain reaction (PCR) that identifies VCG 0421, VCG 0425, and SMV 4 isolates as a group (Southwood et al., 2012b). Isolates that gave a positive result in the IR-SCAR PCR test were further analysed with two RAPD primers that each yield one PCR amplicon that is specific for VCG 0425 isolates, thus differentiating it from VCG0421 and SMV4. DNA extraction and PCR amplifications with the IR-SCAR primers (Hansec-1F, Hansec-2R, HTH-1F and HTH-2R) and RAPD primers (OPA-05 and OPI-18) were conducted as described by Southwood et al. (2012b).