Pathogenicity of fungi isolated from atypical skin blemishes on potatoes in South Africa and Zimbabwe

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Abstract

Potato skin blemishes with known causes (typical blemishes) are manageable compared to those with unknown causes (atypical blemishes). This study investigated possible fungal causal agents of atypical tuber blemishes in South Africa and Zimbabwe through isolation and pathogenicity testing. Several fungal genera were isolated from elephant hide, corky cracks, star-shaped lesions, circular corky lesions, raised corky spots, enlarged lenticels and russetting. Koch's postulates were fulfilled for binucleate *Rhizoctonia* (BNR) AG A, *Macrophomina phaseolina* and *Rhizoctonia solani* AG 3-PT. BNR AG A isolates from South African tubers of cv. Up-to-Date reproduced circular and linear defects on tubers. Koch's postulates could not be fulfilled with the remaining isolates, as re-isolation proved impossible. *M. phaseolina* isolates inoculated on South African cv. Mondial reproduced dark, circular lesions around lenticels conforming to typical symptoms of charcoal rot on potato tubers. *R. solani* AG 3-PT isolates reproduced corky cracks with elephant hide and black scurf with similar aggressiveness on cvs Mondial (South Africa) and Diamond (Zimbabwe). This is the first report of charcoal rot caused by *M. phaseolina,* and of tuber defects caused by BNR AG A on potato in South Africa. This is also the first report of tuber cracking and elephant hide caused by *R. solani* AG 3-PT on potato in Zimbabwe. The findings from this work are crucial in formulating control strategies against these pathogens in order to improve potato tuber quality on the market.

Keywords: Potato; atypical blemishes; binucleate *Rhizoctonia* AG A; *Rhizoctonia solani* AG 3-PT; *Macrophomina phaseolina*

Introduction

Potato is a staple dietary vegetable produced in more than 150 countries in the world. According to Food and Agriculture Organisation statistics (FAOSTATS 2014), there has been a marked increase of 94.6% in potato production in the last decade, especially in the third world countries where total production surpassed that of the developed world in 2005 (FAOSTATS 2014). The high yielding potential and nutritional status of potato makes it a promising crop in providing food security in the face of rising population and reduction in arable land. South Africa, which ranks fourth in potato production in Africa after Algeria, Egypt and Malawi (FAOSTATS 2014), recorded an increase in production of 33% in the last 15 years. The crop contributes approximately 60% of the gross value of vegetables in South Africa (Potatoes South Africa (PSA) 2015). In Zimbabwe, potato is the third most important food crop after maize and wheat (Svubure et al. 2015). Approximately 3500 ha are under potato

production annually in Zimbabwe (FAOSTATS 2014). Recently, the crop has been assigned a national strategic food security crop status as many farmers in Zimbabwe are taking up potato production (Svubure et al. 2015).

The potato industry faces challenges related to disease pressure that impact negatively on the quality of tubers. The crop is susceptible to more than 100 pathogens of which more than 40 are soil borne pathogens (Fiers et al. 2010). Potato tuber blemishes are caused by *Rhizoctonia solani*, *Spongospora subterranea* f. sp. *subterranea*, *Helminthosporium solani*, *Colletotrichum coccodes*, *Fusarium* species and *Streptomyces* species *inter alia* (Nærstad et al. 2012). These pathogens have been associated with black scurf, powdery scab, silver scurf, black dot, dry rot and common scab symptoms on tubers resulting in reduced yield and quality (Tsror et al. 1999; Nærstad et al. 2012). Potato tuber blemishes are also caused by viruses and nematodes (Holgado et al. 2009; Carnegie and McCreath, 2010; Carnegie et al. 2010). They are of concern to the seed potato industry as they re-infect daughter tubers of plants produced from contaminated seed. Superficial blemishes also offer entry points for opportunistic pathogens that may lead to tuber rotting. They increase tuber skin permeability and increase water loss resulting in shrivelling of produce in storage (Nærstad et al. 2012).

The well documented potato blemishes (typical) and a number of unknown (atypical) superficial tuber blemishes contribute to major economic losses (Gherbawy and Gashgari 2013). The loss is worsened by an increase in demand for high quality washed ware potatoes on the market (Tsror et al. 1999; Fiers et al. 2010; 2012). This is because potato tuber colour, size and external appearance are the most relevant tuber attributes determining consumer decisions in potato purchasing (Jemison Jr. et al. 2008; Boesch 2012). In addition, blemished tubers are difficult to peel resulting in high costs incurred in the processing industry (Gherbawy and Gashgari 2013).

Correct disease and pathogen identification is the first vital step to successful control of diseases (Srinivasa et al. 2012). Misdiagnosis followed by incorrect control recommendations can be a waste of time and resources leading to further plant loss (Wang et al. 2012). Rapid diagnosis of pathogens becomes easier when associated with well-known symptoms. This is not the case with atypical potato tuber blemishes. The causal agents of atypical skin blemishes have not yet been determined and there is no consensus in their present symptom terminology. Fiers et al. (2010) classified superficial atypical potato blemishes into corky cracks, corky spots or "rhizoscab", star-like corky lesions and “elephant hide”, of which the latter in the same study is commonly referred to as irregular polygonal sunken corky lesions. Knowledge of the causal agents of these blemishes and their biology is paramount in formulating effective and practical management tactics and enable the incorporation of atypical tuber blemishes in potato seed certification schemes. This will reduce their impact on the quality of produce (Nærstad et al. 2012). A preliminary survey of tuber blemishes showed that fungal pathogens are the primary causal agents of tuber blemishes in South Africa (results not shown). Therefore, the objective of this study was to determine the fungal causal agents of atypical potato skin blemishes in South Africa and Zimbabwe through 1) isolation of fungal microorganisms from blemished tubers collected from different growing regions of the two countries, 2) identification of isolates using classical and DNA-based methods and 3) pathogenicity testing with isolates to fulfil Koch’s postulates.

Materials and Methods

Collection of tuber samples

Potato tubers displaying different skin blemishes were received from potato growing regions in South Africa and Zimbabwe. In South Africa, the tubers were collected in 2013 in collaboration with Potatoes South Africa regional managers, Potato Seed Certification officers and by making use of samples sent to the Diagnostic Clinic of the Potato Pathology Programme at the University of Pretoria. Some of the tubers were collected from the local fresh produce market. Zimbabwean tubers were collected in 2014 from the Potato Seed Co-operative in Msasa, Harare. The blemishes were classified according to the country of origin, cultivar and source region (Tables 1 and 2).

Table 1. Blemishes observed on South African tubers

Source (potato-growing region)	Cultivar	Symptoms										
		A	B	C	D	E	F	G	H	I	J	K
Sandveld	Valor	x	x	x	x	x						
	Mondial	x	x									
	BP1						x					
	Avalanche	x	x	x				x				
Gauteng	Mondial	x		x		x		x				
	Up-to-Date		x				x		x			
Mpumalanga	Mondial							x	x	x		
	Flamingo						x					
	Contessa						x		x	x		
Limpopo	Mondial	x	x				x	x		x	x	x
	Valor	x	x	x	x			x			x	
	Lanorma		x					x				
	Eos				x							
	Up-to-Date		x		x				x			
	Sifra	x										
	BP1										x	

x means that the specific cultivar from a specific region had that symptom, where:

A—corky cracks—hard, dry and coarse fissures formed on tuber surface (Fig. 3) (Fiers et al. 2010; Muzhinji et al. 2014)

B—elephant hide—dark polygonal lesions on the surface of the tuber (Fig. 3) (Fiers et al. 2010; Muzhinji et al. 2014, 2015)

C—black scurf—*Rhizoctonia solani* sclerotia deposited on the surface of the tuber (Fig. 3) (Fiers et al. 2010: Muzhinji et al. 2015)

D—star-like corky lesions—hard, dry, star-shaped injuries on surface of tubers (Fiers et al. 2010)

E—trumpet hole—a narrow funnel-shaped vent into the flesh of potato tuber (Muzhinji et al. 2015)

F—russet—rough skin of potato tuber

G—tuber defects—circular or linear deflections or malformations on the tuber as shown in Fig. 1

H—enlarged lenticels—whitish pores on potato surface that are swollen, giving the tuber a bumpy appearance

I—discoloured sunken lesions—darkened wounds with depressed area in the middle

J—circular corky lesions—wound with hard and dry necrotic tissue that is round in appearance as shown in Fig. 2a, b

K—silver scurf—superficial silvery lesions on the surface of the tuber due to *Helminthosporium solani* infection (Nærstad et al. 2012)

Table 2. Blemishes observed on Zimbabwean tubers

Source (potato-growing region)	Cultivar	Symptoms					
		A	B	C	D	E	F
Nyanga	Amethyst	x	x			x	
	BP1		x		x	x	
	Diamond	x	x	x	x	x	
	KY20					x	
Mazowe	BP1					x	x
Karoi	Amethyst						x
Macheke	BP1						x

x means that a specific cultivar from a specific region had that symptom, where:

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B—elephant hide—dark polygonal lesions on the surface of the tuber (Fig. 3) (Fiers et al. 2010; Muzhinji et al. 2014, 2015)

C—black scurf—*Rhizoctonia solani* sclerotia deposited on the surface of the tuber (Fig. 3) (Fiers et al. 2010; Muzhinji et al. 2015)

D—star-like corky lesions—hard, dry, star-shaped injuries on surface of tubers (Fiers et al. 2010)

E—russet—rough skin of potato tuber

F—raised corky lesions—small, hard and dry wounds on surface of tuber with an elevated appearance

Isolation of fungi associated with tuber skin blemishes

Fungal organisms were isolated from the tuber blemishes following the method of Fiers et al. (2010), with minor modifications. Briefly, the tubers were washed under running tap water and air dried on the laboratory bench. Potatoes from each region were grouped according to type of symptoms, such as corky cracks, corky spots, star-like corky lesions, tuber defects, discolorations, russet, enlarged lenticels and black scurf. Sub-samples of four potatoes from each symptom group were chosen randomly for isolations.

Systematic isolation was done by aseptically excising approximately 0.25 cm^2 pieces from the margins of infected parts of the tuber. The excised diseased plant tissues were surface sterilised in 1% sodium hypochlorite (NaOCl) solution for 1 min, rinsed three times in sterile distilled water and blot dried. Subsequently, they were plated individually onto Water Agar (WA) (Biolab) amended with 300 mg l^{-1} of streptomycin-sulphate (Sigma-Aldrich) and incubated at 25 ºC for 3 days. Fungal colonies were purified twice by subculturing onto 90 mm Potato Dextrose Agar (PDA) (Biolab) plates containing 300 mg l^{-1} of streptomycin-sulphate. The isolates were stored by cryopreservation, on agar slants and in sterile water.

Fungal isolates identification

Fungal isolates were described in terms of their form, colour, size and elevation on growth media. Microscopic examination of fungal structures was performed while referring to identification keys and manuals (Mathur and Kongsdal 2001; Leslie and Summerell 2006).

Molecular identification was done by sequencing the ITS region of rDNA to confirm classical fungal identities. DNA extraction from pure cultures was carried out using the Zymo Research (ZR) Soil Microbe DNA Miniprep™ kit (The Epigenetics Company, USA) according to manufacturer's instructions. DNA quantity was measured using a Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies, Rockland, USA). The universal primers ITS 1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al. 1990) were used in the PCR reactions. Amplification was done in 25 µl PCR reaction mixture comprising of 160 mM 10 x NH_4 reaction buffer (Bioline, London, UK), 3 µM $MgCl_2$, 250 µM of each dNTP (Bioline), 0.2 µM of each primer, 0.25 U BIO*Taq*™ DNA polymerase (Bioline) and 1 µl DNA template. The PCR reactions in a thermal cycler (Bio-Rad) included an initial denaturation step at 95 ºC for 5 min and 35 cycles of 95 ºC for 50 s, 56 ºC for 50 s and 72 ºC for 1 min. The quality of amplicons was checked by running a 1% (w/v) agarose gel electrophoresis before purification of the remaining PCR product with Sephadex® G-50 gel (Sigma-Aldrich) according to manufacturer's guidelines. The sequencing reactions for each primer were performed using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) following the manufacturer's instructions. The sequences of amplicon forward and reverse strands were determined using the ABI PRISM 3500xl model Genetic Analyser (Applied Biosystems) at the DNA sequencing facility, University of Pretoria. The identity of each fungal isolate was obtained by standard nucleotide blasting of consensus sequences created from forward and reverse strands (BioEdit v 7.2.5) against the GenBank database (http://www.ncbi.nlm.nih.gov/genbank). The sequences of pathogenic isolates were deposited in GenBank.

Pathogenicity tests

At most four representative fungal isolates of each species were used in pathogenicity tests, following the procedure by Fiers et al. (2010), with modifications. Fungal inoculum was prepared by sterilising 200 g of wheat bran medium moistened with 60 ml of distilled water in 1-l Schott bottles for 1 h at 121 ºC. The same bottles were autoclaved again for 30 min on the following 2 consecutive days and left for 4 days at room temperature to release toxic compounds. Each bottle of media was inoculated with ten 6 mm diameter plugs of 10-day old fungal isolate colonies grown on PDA. The bottles were incubated at ± 25 ºC for 3 weeks with regular shaking to promote uniform colonization of medium.

Twenty-five grams of fungal inoculum was used to inoculate steam-sterilised soil (27% clay, 7% silt and 66% sand) in 20-cm diameter plastic pots. One certified (according to the South African Potato Certification Scheme; DAFF 2010), visually clean and sprouted seed tuber of the same cultivars as the cultivar of isolate origin was planted in each pot. The experiment was laid out in a completely randomized design (CRD) with four repeats. Control pots were inoculated with 25 g of sterile wheat bran. The pots were kept in a greenhouse at ± 21 °C, 12 h photoperiod and watered every second day. Fertilization was done once per fortnight with Multifeed nutrient solution (3:1:6 NPK) (AECI Ltd, Witfield, SA) to facilitate plant growth.

Progeny tubers were harvested at 120 days after emergence, washed and scored for blemish incidence and severity. Disease severity scoring for corky cracks and elephant hide was done using a 0 – 5 scale by Carling and Leiner (1990) with modifications, where 0 = no blemish present on tuber, 1 = less than 1% of the tuber surface area covered with blemishes, 2 = 1 to 10% of the tuber surface area covered with blemishes, 3 = 11 to 20% of the tuber surface area covered with blemishes, 4 = 21 to 50% of the tuber surface area covered with blemishes and 5 = 51% or more of the tuber surface area covered with blemishes. The disease index (DI) for corky cracks and elephant hide was calculated using the following formula: $DI = \{[0(n_0) + 0.25(n_1) + 0.5(n_2) + 0.75(n_3) + 1(n_4)]/ (N_{total})\} \times 100$ where n_x corresponded to the number of tubers in the x rating category per replicate and N was the total number of tubers rated per replicate (Muzhinji et al. 2015). A disease severity scale of 0 – 3 by Nærstad et al. (2012), where 0 represented no blemish on tubers, 1 = < 30%, 2 = 30 – 60% and 3 = > 60% of tuber covered with blemishes, was used for the remainder of the blemishes . DI for these blemishes was calculated by modifying the formula by Chowdhury et al. (2014) and Yang et al. (2015a) as follows: $DI = [(0n_0 + 1n_1 + 2n_2 + 3n_3) / 3N] \times 100$, with variables as described by Muzhinji et al. (2015). To fulfil Koch's postulates, re-isolations were performed from progeny tubers showing similar blemishes to the original symptoms harvested from both inoculated and control pots by repeating the same protocols as described above.

Data analysis

Results from pathogenicity tests were presented in tabular form to compare blemishes on progeny tubers with those on tubers of origin. The data for percentage disease index were arc sin square root transformed and subjected to analysis of variance (ANOVA) using the Statistical Analysis Software (SAS) version 9.3 for Windows (SAS Institute 2010). Multiple treatment comparisons were performed using Fisher's Least Significant Difference (LSD) to determine differences in aggressiveness of isolates in causing a certain blemish.

Results

Fungal species identified

A total of 166 fungal isolates were obtained from various atypical tuber blemishes in South Africa and Zimbabwe. Isolates were sorted according to species, source cultivar and country of origin. Finally, 43 fungal species were used in pathogenicity tests (Table 3). The most frequently isolated fungal genus was *Fusarium*, which constituted almost 50% of the species used in pathogenicity tests in this study. *F. oxysporum* was isolated from all cultivars except Amethyst and around 43% of all *Fusarium* isolates tested in this study caused dry rot on progeny tubers. *Alternaria*, *Bionectria*, *Chaetomium*, *Colletotrichum*, *Curvularia*, *Macrophomina*, *Nigrospora*, *Phoma*, *Phomopsis*, *Plectosphaerela*, *Pythium* and *Rhizoctonia* species were also associated with tuber blemishes in South Africa and Zimbabwe. Common saprophytes like *Mucor*, *Penicillium* and *Rhizopus*; potential biological control agents such as *Trichoderma*; and several yeast species were also part of the superficial tuber mycobiota but were not included in pathogenicity tests. The soil-borne pathogen of potatoes, *R. solani* AG 3-PT was prevalent on tubers from both countries .

Table 3. Fungal species isolated from and inoculated onto the same cultivars in pathogenicity tests

Fungal species	Cultivar								
	Mondial	**Up-to-Date**	**Valor**	**BP1**	**Lanorma**	**Sifra**	**Eos**	**Diamond**	**Amethyst**
Fusarium oxysporum	x	x	x	x	x	x	x	x	
F. chlamydosporum	x	x			x				
F. succisae					x		x		
F. brachygibbosum	x	x			x				
F. redolens	x		x						
F. proliferatum	x								
F. solani			x						
F. falciforme	x	x		x					
F. thapsinum	x								
F. acutatum	x								
F. acuminatum	x								
F. equiseti	x		x						x
F. incarnatum			x						
F. verticilloides			x						
F. keratoplasticum			x						
F. nygamai			x						
F. nelsonii									x
Rhizoctonia solani AG 3-PT	x	x		x				x	x*
R. solani AG4			x						x
Binucleate *Rhizoctonia* AG A		x							
Alternaria arborescens					x				
A. solani	x								
A. tenuissima	x					x			
A. alternata		x		x					
Nigrospora oryza									x
Mortierella alpine	x								
Macrophomina phaseolina	x	x		x					x
Chaetomium globosum		x						x	
Chaetomium aureum			x					x	
Chaetomium subspirilliferum								x	
Curvularia lunata									x
Curvularia trifolii								x	
Colletotrichum coccodes	x	x					x	x	
Pythium irregulare	x								
Phoma tropica									x
Phoma multirostrata		x							
Phomopsis columnaris	x								
Phaeocytostroma ambiquum	x								
Plectosphaerella cucumerina		x							
Setophoma terrestris	x	x	x						
Sordaria tomento-alba	x	x							
Neocosmopara vasinfecta		x							
Bionectria ochroleuca				x					

The second sentence does not refer to the blank cells but an asterisk in the table showing that *Rhizoctonia solani* AG 3-PT that was isolated from cultivar Amethyst could not be inoculated onto the same cultivar in pathogenicity tests because of poor quality seed that failed to germinate.

Pathogenicity tests

The blemishes observed on progeny tubers were described in comparison to the original blemishes on source tubers (Tables 4 and 5). Tables 4 and 5 show that the majority of fungal species used in pathogenicity tests failed to reproduce the blemishes of origin on progeny tubers. A few produced these symptoms but the control tubers from non-inoculated pots had the same blemishes, which nullified these species as causative agents. *Fusarium oxysporum* frequently reproduced corky cracks on different cultivars but these cracks also appeared in control tubers. The recovery of inoculated isolates from blemished progeny tubers was the last condition to confirm that the isolates were involved in causing blemishes. The majority of fungal species could not be re-isolated from the blemishes. For instance, *P. irregulare* on Mondial and *F. succisae* on Eos reproduced the original symptoms but could not be re-isolated from the blemishes.

Table 4. Fungal species used in South African pathogenicity tests, showing symptoms observed on original tubers and on progeny tubers

Isolate	Species	Cultivar	Symptoms			Isolate recovery
			Original symptoms	Similar to original	Different from original	
8F36	*Fusarium brachygibbosum*	Up-to-Date	Circular defects	Circular defects	Silver scurf, black dot	–[a]
8F45	*Neocosmopara vasinfecta*	Up-to-Date	Linear and circular defects on tubers	Linear and circular defects on tubers	Silver scurf, black dot	–[a]
8F47	*Sordaria tomento-alba*	Up-to-Date	Linear and circular defects on tubers	Linear and circular defects on tubers	Silver scurf, black dot	+[a]
12F10	BNR AG A (repeat)	Up-to-Date	Linear and circular defects on tubers	Linear and circular defects on tubers	Enlarged lenticels, minor cracking, trumpet hole with elephant hide, small aerial tubers	+
11F1	*Setophoma terrestris*	Up-to-Date	Russet, elephant hide		Enlarged lenticels, silver scurf, linear defects	–[a]
11F13	*Alternaria solani*	Up-to-Date	Russet		Enlarged lenticels, silver scurf, circular defects	–[a]
11F15	*Phoma multirostrata*	Up-to-Date	Russet, star-like corky lesions		Severe dark polygonal necrotic lesions, enlarged lenticels, secondary growth, linear and circular defects	–[a]
11F16	*Plectosphaerella cucumerina*	Up-to-Date	Russet, star-like corky lesions		Enlarged lenticels	–
11F20	*Macrophomina phaseolina*	Up-to-Date	Elephant hide, star-like corky lesions		Enlarged lenticels, silver scurf, black dot, microsclerotia, secondary growth	+
11F22	*Colletotrichum coccodes*	Up-to-Date	Elephant hide, russet		Enlarged lenticels, silver scurf, black dot, circular and linear defects	–[a]
11F28	*F. oxysporum*	Up-to-Date	Elephant hide, russet		Enlarged lenticels, circular and linear defects	–[a]
11F29	*F. chlamydosporum*	Up-to-Date	Elephant hide, star-like corky lesions, enlarged lenticels		Russet, silver scurf, black dot, linear and circular defects	–[a]
11F30	*Chaetomium globosum*	Up-to-Date	Elephant hide, star-like corky lesions		Enlarged lenticels, russet, linear and circular defects	–[a]
Control		Up-to-Date			Linear and circular defects, silver scurf, black dot, enlarged lenticels, russet	–[a]

Isolate	Species	Cultivar	Symptoms			Isolate recovery
			Original symptoms	Similar to original	Different from original	
5F2	*F. brachygibbossum*	Mondial	Elephant hide and deep cracking		Russet, enlarged lenticels	−
5F8	*Bionectria ochroleuca*	Mondial	Elephant hide and deep cracking		Russet, enlarged lenticels	−
5F9	*Pythium irregulare*	Mondial	Elephant hide and deep cracking	Cracking	Russet, enlarged lenticels	−
5F10	*Mortierella alpine*	Mondial	Elephant hide and deep cracking		Russet, enlarged lenticels	−
7F2	*Rhizoctonia solani* AG3-PT	Mondial	Black scurf	Severe black scurf	Russet, dark enlarged lenticels, cracking, elephant hide	+
7F4	*Phaeocytostroma ambiquum*	Mondial	Misshapen tubers, silver scurf		Russet, enlarged lenticels	−
7F6	*F. acuminatum*	Mondial	Tuber defects and elephant hide		Russet, enlarged lenticels	−
7F11	*Macrophomina phaseolina*	Mondial	Circular corky lesions, tuber defects, elephant hide	Circular necrotic lesions	Dark circular lesions around lenticels, unusually large and discoloured lenticels developing into circular lesions, russet, secondary growth, sclerotia	+
7F19	*S. terrestris*	Mondial	Misshapen tubers, silver scurf		Russet, enlarged lenticels	−
7F21	*F. proliferatum*	Mondial	Tuber defects, elephant hide		Russet, enlarged lenticels, black dot, dry rot, minor cracking	−
7F22	*Phomopsis columnaris*	Mondial	Discoloured sunken lesions		Russet, enlarged lenticels	−
7F31	*F. thapsinum*	Mondial	Tuber defects, black scurf		Russet, enlarged lenticels, dry rot	+
7F34	*F. acutatam*	Mondial	Misshapen tubers, silver scurf		Enlarged lenticels	−
8F12	*A. tennuisima*	Mondial	Corky cracks		Russet, enlarged lenticels	−
8F16	*S. tomento-alba*	Mondial	Corky cracks		Russet, enlarged lenticels	−
8F19	*F. incarnatum*	Mondial	Corky cracks		Russet, enlarged lenticels	−
8F24	*F. chlamydosporum*	Mondial	Corky cracks		Russet, enlarged lenticels	−
8F38	*F. equiseti*	Mondial	Corky cracks		Russet, enlarged lenticels, dry rot	+
Control		Mondial			Russet, enlarged lenticels	−
7F15	*S. terrestris*	Valor	Tuber defects, black scurf		Russet, enlarged lenticels	−
7F16	*F. incarnatum*	Valor	Tuber defects, black scurf		Russet, enlarged lenticels	−
7F24	*F. redolens*	Valor	Tuber defects, black scurf		Russet, enlarged lenticels	−
7F28	*F. verticilloides*	Valor	Tuber defects, black scurf		Russet, enlarged lenticels	−
7F31	*F. thapsinum*	Valor	Tuber defects, black scurf		Russet, enlarged lenticels, dry rot, common scab	+
7F33	*F. solani*	Valor	Circular corky lesions		Russet, enlarged lenticels, dry rot	+
7F36	*F. nygamai*	Valor	Tuber defects, black scurf		Russet, enlarged lenticels, dry rot, common scab	+
8F34	*A. alternata*	Valor	Star-like corky lesion		Russet, enlarged lenticels, brown lesions, common scab	−
8F51	*F. keratoplasticum*	Valor	Star-like corky lesion		Russet, enlarged lenticels, dry rot	−

Isolate	Species	Cultivar	Symptoms			Isolate recovery
			Original symptoms	Similar to original	Different from original	
8F52	*F. equiseti*	Valor	Tuber defects, elephant hide		Russet, enlarged lenticels, dry rot	+
Control					Russet, enlarged lenticels	−
8F11	*A. alternata*	Lanorma	Tuber defects, elephant hide		Enlarged lenticels, malformation	−
8F13	*A. aborescens*	Lanorma	Tuber defects, elephant hide		Enlarged lenticels	−
8F35	*F. brachygibbosum*	Lanorma	Tuber defects, elephant hide		Enlarged lenticels, secondary growth	−
8F39	*F. oxysporum*	Lanorma	Tuber defects, elephant hide		Enlarged lenticels, malformation	−
8F43	*F. succisae*	Lanorma	Tuber defects, elephant hide		Enlarged lenticels, russet, dry rot	+
8F55	*F. chlamydosporum*	Lanorma	Tuber defects, elephant hide		Enlarged lenticels	−
Control		Lanorma			Russet, enlarged lenticels, malformation, secondary growth	−
8F40	*F. succisae*	Eos	Star-like corky cracks	Star-like corky lesions	Enlarged lenticels, russet, dry rot	+
8F50	*F. oxysporum*	Eos	Star-like corky cracks	Star-like lesions	Cracking, enlarged lenticels, dry rot	+
8F60	*C. coccodes*	Eos	Misshapen tuber		Enlarged lenticels, black dot	+
Control		Eos			Enlarged lenticels, russet	—
7F12	*M. phaseolina*	BP1	Circular corky lesions		Russet, discoloured enlarged lenticels	+
7F20	*F. falciforme*	BP1	Circular corky lesions		Russet, enlarged lenticels	−
7F35	*F. oxysporum*	BP1	Circular corky lesions, misshapen tubers		Enlarged lenticels, star-like lesions, dry rot and cracking	+
Control		BP1			Russet, enlarged lenticels, cracking	—
7F35	*F. oxysporum*	Sifra	Deep, discoloured corky cracks		Enlarged lenticels, star-like lesions	−
8F32	*A. tenuissima*	Sifra	Tuber defects		Russet, enlarged lenticels	−
Control		Sifra			Russet, enlarged lenticels	—

+ or − signs (in the isolate recovery column) mean that the specific isolate was re-isolated or not re-isolated from progeny tubers, respectively

[a]Binucleate *Rhizoctonia* AGA was isolated from tuber defects on progeny tubers of cv. Up-to-Date harvested from pots inoculated with these isolates and control treatments

Table 5. Fungal species used in Zimbabwean pathogenicity tests, showing symptoms observed on original tubers and on progeny tubers

Isolate	Species	Cultivar	Symptoms			Isolate recovery
			Original symptoms	Similar to original	Different from original	
10F2	*Chaetomium aureum*	Diamond	Deep corky crack, black scurf		Russet, dark enlarged lenticels, silver scurf, secondary growth, black dot	−
10F6	*Fusarium oxysporum*	Diamond	Deep corky crack, star-like corky lesions		Discoloured enlarged lenticels, silver scurf	−
10F7	*Chaetomium globossum*	Diamond	Severe russet	Corky rough skin	Discoloured lenticels, black dot, silver scurf	+
10F14	*Colletotrichum coccodes*	Diamond	Deep corky crack, star-like corky lesions		Silver scurf and black dot, enlarged lenticels	+
10F16	*Epicoccum nigrum*	Diamond	Severe russet	Russet	Silver scurf, discoloured enlarged lenticels	−
10F26	*Curvularia trifolii*	Diamond	Deep corky crack, elephant hide		Silver scurf, discoloured lenticels	−
10F29	*Rhizoctonia solani* AG3 PT	Diamond	Deep corky crack, black scurf	Severe black scurf, cracking	Discoloured enlarged lenticels, elephant hide	+
10F31	*R. solani* AG3 PT	Diamond	Deep corky crack, black scurf	Black scurf, cracking	Silver scurf, discoloured enlarged lenticels	+
10F35	*Chaetomium subspirilliferum*	Diamond	Deep corky crack, black scurf		Severe russet, black dot, dark enlarged lenticels	−
Control		Diamond			Silver scurf, black dot, enlarged lenticels	−
10F8	*F. nelsonii*	Amethyst	Raised corky spots		Enlarged lenticels	−
10F10	*F. oxysporum*	Amethyst	Raised corky spots	Shallow cracks	Enlarged lenticels, russet	−
10F15	*Nigrospora oryza*	Amethyst	Raised corky spots		Russet, enlarged lenticels, silver scurf	−
10F21	*Phoma tropica*	Amethyst	Raised corky spots		Deep cracks, enlarged lenticels, russet	−
10F24	*Curvularia lunata*	Amethyst	Raised corky spots		Dark enlarged lenticels	−
10F30	*R. solani* AG4	Amethyst	Raised corky spots		Enlarged lenticels, rot on green exposed parts	−
Control		Amethyst			Cracking, russet	

+ or − signs (isolate recovery column) mean that the specific isolate was re-isolated or not re-isolated from progeny tubers, respectively

Fusarium brachygibbosum, *Neocosmopara vasinfecta* and *Sordaria tomento-alba* reproduced the observed circular and linear defects on tubers of cv. Up-to-Date. *S. tomento-alba* was recovered from progeny tubers. However, tuber defects were noted on Up-to-Date progeny tubers harvested from control pots and on those inoculated with fungal species originally from different blemishes, such as *A. solani, Phoma multirostrata* and *S. terrestris*. Amongst other fungal species, binucleate *Rhizoctonia* Anastomosis Group (BNR AG) A was commonly recovered after re-isolations from the blemished progeny tubers in this cultivar. It was suspected that BNR AG A came from other sources such as latently infected seed tubers. A repeat of the experiment was done with BNR AG A isolates 12F10, 12F25 and 12F35 (GenBank accession numbers KU721994, KU721996,

KU721997 respectively). The isolates reproduced linear and circular defects on progeny tubers (Fig. 1) with varying degrees of severity (P = 0.003) and mean disease index (DI) ranging from 42.50 to 46.75% (Table 6). BNR AG A was re-isolated from the blemished tubers and not from controls.

Fig. 1. Linear and circular tuber defects caused by binucleate *Rhizoctonia* AG A

Table 6. Mean percentage disease index of different blemishes caused by fungal isolates on cultivars of origin

Isolate	Cultivar	Accession number	Symptom		
			Tuber defects	Charcoal rot	Cracking and elephant hide
12F35 (BNR AG A)	Up-to-Date	KU721997	46.75 (0.47) a	N/A	N/A
12F10 (BNR AG A)	Up-to-Date	KU721994	45.75 (0.46) a	N/A	N/A
12F25 (BNR AG A)	Up-to-Date	KU721996	42.50 (0.43) a	N/A	N/A
7F11 (*M. phaseolina*)	Mondial	KU161137	N/A	34.78 (0.63) a	N/A
7F13 (*M. phaseolina*)	Mondial	KU161139	N/A	15.08 (0.34) b	N/A
7F12 (*M. phaseolina*)	BP1	KU161138	N/A	11.13 (0.29) bc	N/A
7F2 (*R. solani* AG 3-PT)	Mondial	KU161133	N/A	N/A	40.35 (0.69) a
10F29 (*R. solani* AG 3-PT)	Diamond	KU161142	N/A	N/A	39.68 (0.68) a
10F31 (*R. solani* AG 3-PT)	Diamond	KU161134	N/A	N/A	36.65 (0.65) a
Control			1.50 (0.02) b	0.00 (0.00) c	2.48 (0.11) b
P value			0.003	0.0019	<0.0001
LSD			0.363	0.268	0.153
SEM			0.074	0.069	0.067

Means followed by the same letter in a column are not statistically different. *BNR AGA*—binucleate *Rhizoctonia* AG A, *M. phaseolina*—*Macrophomina phaseolina*, *R. solani* AG 3-PT—*Rhizoctonia solani* AG 3-PT. The transformed means are presented in parenthesis

N/A means that the isolate was not responsible for causing that particular symptom

In addition to BNR AG A, *M. phaseolina* isolates 7F11 and 7F13 (GenBank accession numbers KU161137 and KU161139 respectively) isolated from corky circular lesions on Mondial tubers from the Limpopo growing region caused tuber charcoal rot. Progeny tubers had circular corky lesions (Fig. 2a), sclerotia similar to black scurf of *R. solani* and ashy grey enlarged lenticels. Dark necrotic lesions on some tubers developed around lenticels resembling dry rot of *Fusarium* (Fig. 2b). The blemishes had a mean DI of 34.78% and 15.08% for isolates 7F11 and 7F13, respectively. *M. phaseolina* was recovered from these blemishes thereby fulfilling

Koch's postulates. There were cultivar differences in susceptibility to *M. phaseolina* (P = 0.0019). Progeny tubers of cv. BP1 harvested from pots inoculated with isolate 7F12 (KU161138) had discoloured lenticels only.

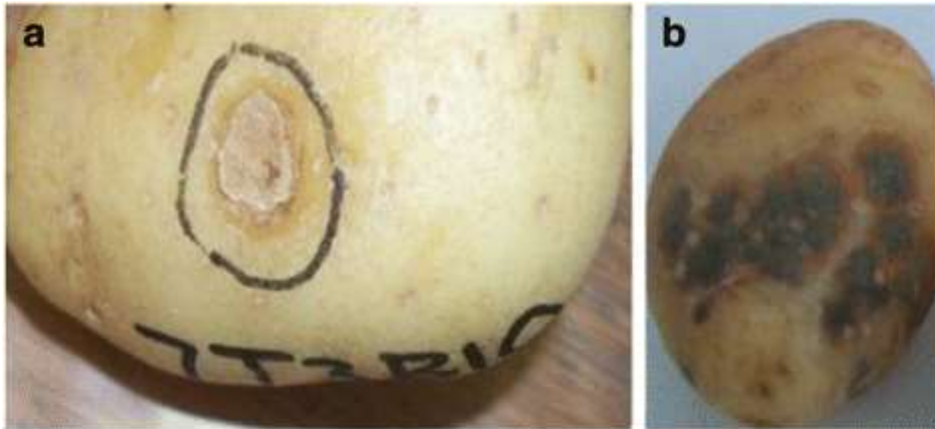

Fig. 2. Charcoal rot caused by *Macrophomina phaseolina.***a** Circular corky lesions; **b** dark circular lesions referred to as the dry rot stage of the disease

Rhizoctonia solani AG 3-PT isolate 7F2 (KU161133) originated from cv. Mondial of South Africa while isolates 10F29 and 10F31 (KU161142 and KU161134) were isolated from cv. Diamond of Zimbabwe. The isolates were obtained from corky cracks with black scurf. The progeny tubers of these cultivars had severe black scurf, cracking, elephant hide and enlarged discoloured lenticels (Fig. 3). A disease index of up to 40.35% for cracking and elephant hide blemishes was recorded. Both Zimbabwean and South African *R. solani* AG 3-PT isolates had similar aggressiveness on the two potato cultivars (Table 6). The pathogen was recovered from more than 80% of the infected progeny tubers and not from controls.

Fig. 3. Corky cracks with elephant hide caused by *Rhizoctonia solani* AG 3-PT

Chaetomium globosum isolate 10F7 (KU161140) was obtained from severely russetted tubers of cv. Diamond (Fig. 4). The daughter tubers had corky rough skin and the isolate was recovered from these blemishes. *C. globosum* could, however, not be considered a potential pathogen of potato in this study because only one isolate was tested. Further work has to be done to confirm these findings.

Fig. 4. Russet symptoms on the surface of cv. Diamond from which *Chaetomium globosum* was isolated

Another striking observation was the presence of silver scurf and black dot (Fig. 5) on progeny tubers of cvs Up-to-Date and Diamond. The blemishes were present on almost all progeny tubers including control progeny tubers of these cultivars regardless of the fact that *Helminthosporium solani* and *Colletotrichum coccodes* were not inoculated in the respective pots (Tables 4 and 5). This may be due to seed borne inoculum of these pathogens. *C. coccodes* was isolated from several blemishes including corky cracks (Fig. 3), misshapen tubers, russet (Fig. 4) and enlarged lenticels (Fig. 6). When corky lesions are present on tubers these lesions can mask the presence of silver scurf and black dot. *H. solani* is difficult to isolate and its growth is slow compared to other fungi, which may explain why only *C. coccodes* was isolated from progeny tubers. Although *C. coccodes* did not reproduce symptoms of origin, it caused black dot on cvs Eos and Diamond.

Fig. 5. Silver scurf and black dot observed on progeny tubers of cvs Up-to-Date and Diamond

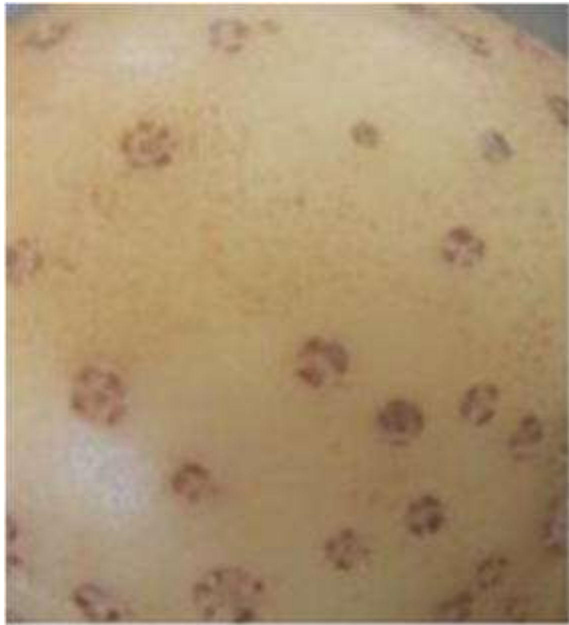

Fig. 6. Enlarged lenticels from which *Colletotrichum coccodes* was isolated

Discussion

The term 'atypical tuber blemishes' was first used by Fiers et al. (2010), to describe potato skin blemishes with unknown causes. In this study, the blemishes identified from different growing areas of South Africa and Zimbabwe included corky cracks, elephant hide, circular corky lesions, star shaped corky lesions, linear and circular defects, russet and enlarged lenticels. Similar blemishes have been reported on potato tubers in Europe (Fiers et al. 2010, 2012) and Asia (Gherbawy and Gashgari 2013). These blemishes reduce quality of the marketable produce since they are cosmetic in nature resulting in rejection by consumers (Rukaia et al. 2013).

Potato skin blemishes may be of biotic or abiotic origin. The current study focused on establishing the fungal biotic causes of atypical blemishes through fulfilling Koch's postulates. The isolate had to incite the original symptoms and should be re-isolated from these symptoms for it to be considered the causal agent of the blemish (Lipkin 2008). Most isolates tested in this study failed to fulfil pathogenicity requirements. Some did not cause any symptoms on progeny tubers whilst others produced symptoms similar to those observed in the controls. These non-pathogenic fungal isolates may have existed on tuber surfaces as mere contaminants (Fiers et al. 2010) or as suppressors or synergists of pathogenic isolates (Dianese et al. 2003; Lear and Lewis 2012). *Bionectria ochroleuca, Chaetomium globosum* and *Trichoderma* species frequently isolated from different skin blemishes in this study are potential biological control agents of fungal pathogens (Soytong and Ratanacherdchai 2005; Samaga et al. 2013; Aggarwal et al. 2014). In some cases, the isolates could have been pathogenic but proof of causation was difficult to establish. For instance, when specific environmental factors required to promote symptom development do not prevail (Fiers et al. 2010), the candidates remain latent on plant surfaces. Where a fungal pathogen was not re-isolated from a lesion, it is assumed that it was not the cause of the lesion but it may simply be due to difficulty in retrieving the isolate from a lesion. Alternatively, lesions may be histological modifications to tubers, such as suberization, which may prevent pathogen development.

Overall, the study revealed the causal agents of tuber defects, charcoal rot and corky cracks with elephant hide to be binucleate *Rhizoctonia* AG A, *Macrophomina phaseolina* and *Rhizoctonia solani* AG 3-PT, respectively. *Fusarium* species were most frequently associated with tuber blemishes. These findings were in agreement with Fiers et al. (2010) and Gherbawy and Gashgari (2013) who isolated mostly *Fusarium* species from blemished potatoes in France and Saudi Arabia, respectively. In addition to *Fusarium* species, the same authors isolated *Alternaria*, *Clonostachys, Penicillium*, *Plectosphaerela*, *Rhizoctonia* and *Mucor,* which were also obtained in this study. Tuber cracking, enlarged lenticels and russet were common symptoms observed on harvested progeny tubers. Presence of these blemishes could be attributed to poor soil moisture management during tuber growth. High soil humidity is known to facilitate opening of lenticels (Fiers et al. 2010) while fluctuation of soil moisture during tuber expansion can result in the breaking of the periderm (Libuy 2006). Tuber cracking can also attributed to herbicide damage, infection by *Streptomyces reticuliscabiei* and viruses such as Potato spindle tuber viroid (PSTVd) and Potato mop-top virus (PMTV) (Bouchek-Mechiche et al. 2013). In this study, *F. oxysporum* was often isolated from tuber cracks and enlarged lenticels on both original and progeny tubers. The appearance of these blemishes on control tubers too could mean that other factors were contributing to their occurrence. These findings support claims that *Fusarium* species are saprophytes or opportunistic pathogens on plant surfaces, attacking plant tissues that are weakened by primary pathogens or abiotic stresses (Chehri et al. 2011).

This study provides the first report of BNR AG A causing tuber malformations, which manifest as linear and circular defects. According to Hartill (1989), malformations emanate from either suppression of tissue development by an epiphytic fungus producing growth regulating toxins or uneven growth due to inadequate photosynthates reaching tubers after destruction of stem and stolon phloem vessels by the pathogen. Previously, reports of tuber malformations were associated with *R. solani* AGs. *R. solani* AG 2Nt, a subset of AG 2-1 was associated with severe tuber malformations in New Zealand (Das et al. 2014). BNR AGs were thought to be saprophytic and some were demonstrated to be biological control agents of plant pathogens (Carling and Leiner 1986; Escande and Echandi 1991). However, recent studies have associated BNR AGs with a number of diseases on different plant hosts. BNR AG A was pathogenic on several crops in China (Yang and Wu 2013). Woodhall et al. (2011) isolated an unknown BNR from mild elephant hide symptoms on potato tubers in Great Britain, whilst Miles et al. (2013) reported the ability of AG A to cause disease on potato stems and sugar beet roots in the Pacific Northwest. Yang et al. (2014) demonstrated that BNR AG A, AG K, AG F and AG U were capable of causing stem canker of potato in China. They also characterised BNR AG G and AG W associated with potato stem canker in the subsequent year (Yang et al. 2015a, b). In South Africa, AG A and AG R were reported to cause stem canker and black scurf on potato (Muzhinji et al. 2015). On the contrary, no sclerotia were observed on progeny tubers inoculated with BNR AG A in our study. This could possibly be due to variability in virulence of isolates within the same AG as explained by Muzhinji et al. (2015). In summary, these findings support the increasing importance of BNR AGs in causing potato diseases with varying degrees of severity from region to region.

Macrophomina phaseolina was shown to cause charcoal rot of potatoes in this study. The symptoms observed

on progeny tubers conform to the description of charcoal rot symptoms on potato tubers by Hooker (1981) and Somani et al. (2013). The pathogen enters the tubers through eyes and enlarged lenticels resulting in black spots appearing around the lenticels. Infected lenticels develop into water soaked lesions due to the necrotic effects of vivotoxins asperlin, isoasperlin, phomalactone, phaseolinic acid, phomenon and patulin, the most potent being phaseolinone (Islam et al. 2012; Kumari and Sharma 2013). Similarly to the fusarim of *Fusarium* species, phaseolinone toxin is a threat on ware and processing potatoes as it is thought to suppress innate immunity of both plants and animals (Sett et al. 2000). The lesions turn black at an advanced stage of the disease cycle, normally in storage, resembling dry rot symptoms (Verma and Sharma 1999). The dry rot stage occurs due to formation of dark sclerotia for overwintering in infected tissue that is worsened by low humidity conditions, promoting shrinking of lesions (Arora and Khurama 2004). Secondary infection can result in a foul smell and rotting of the entire tuber. Davis et al. (2014) added that the pathogen can cause darkening of the potato stem (referred to as ashy stem blight) in the field similar to the blackleg symptom caused by *Pectobacterium* and *Dickeya* species (Ngadze et al. 2012; Somani et al. 2013).

Tubers infected with *M. phaseolina* were collected from the fresh produce market in Pretoria and sclerotia were not visible on tuber surfaces. It is common practice by farmers to remove most of these propagules from the tuber surface during the rotary barrel brush washing process to improve skin shine (Peters and Wiltshire 2006). Sclerotia on potato tubers have always been associated with black scurf of *Rhizoctonia solani* (Fiers et al. 2010; Muzhinji et al. 2015). The sclerotia produced by virulent strains of *M. phaseolina* can be confused with those of *R. solani*. The confusion may result in serious disease management implications where growers implement wrong control strategies due to wrong diagnosis.

Furthermore, appearance of charcoal rot on potato results in difficulties in making agronomic decisions. *M. phaseolina* is known to cause charcoal rot on sunflower (Hussain et al. 1990; Bokor 2007), soybeans (Yang and Navi 2005) and cereal crops including maize, wheat, sorghum (Saleh et al. 2009) and sesame (Chowdhury et al. 2014). Growers the world over follow 3 - 4 year rotation schemes alternating these crops with potatoes to break various pathogen life cycles and to replenish soil nitrogen (Peters et al. 2008). The fact that these crops are alternative hosts for charcoal rot may increase disease risk in subsequent seasons. This can be exacerbated by adoption of conservation tillage systems in potato production that prolong debris decomposition and promote high pathogen inoculum density in soil (Mol et al. 1996; Krupinsky et al. 2002).

Charcoal rot on potatoes has been reported in the Central Valley of California (Davis et al. 2014) as well as in Sudan and India, where yield losses of up to 40% were caused (Sahai et al. 1970). To the best of our knowledge, this is the first time the disease has been reported on potato in South Africa. It is common in tropical and sub-tropical regions with soil temperatures exceeding 28 °C particularly during tuber maturation (Hooker 1981; Somani et al. 2013). The Limpopo growing region of South Africa is characterised by very hot summers from August to March. Farmers in Limpopo prefer planting the main potato crop in winter, avoiding high temperatures experienced in summer. However, tuber maturation and harvesting of the winter crop coincide with maximum temperatures of up to 33 °C in August to November (van der Waals et al. 2013), predisposing tubers to infection by *M. phaseolina*. High temperatures are thought to stress and weaken the plants hence

making them more prone to infection (Palti 1981). Charcoal rot disease severity and incidence may increase in future in the face of climate change. Growers must be aware of this potential threat and must begin to change their management practices. The speculation made by van der Waals et al. (2013) for Limpopo farmers to shift potato planting dates from June to April may help avoid high temperatures at harvest.

This study demonstrated for the first time the existence of tuber corky cracks and elephant hide symptoms on potatoes due to *R. solani* AG 3-PT in Zimbabwe. The findings agree with Muzhinji et al. (2014) who published similar results from South Africa. The results conclusively concur with the observation made by Fiers et al. (2010) that *R. solani* AG 3-PT is associated with corky cracks and polygonal lesions. The presence of *R. solani* AG 3-PT in Zimbabwe could be explained by exchange of potato germplasm between South Africa and Zimbabwe. In the last decade, Zimbabwe has relied on South Africa for seed potatoes and seed borne inoculum can eventually establish in the soil of the receiving country. The South African potato seed certification stipulates zero tolerance in case of generation zero (G0) seed for potato fungal pathogens that include *Oospora pustulans*, *R. solani*, *Spongospora subterranea* f.sp. *subterranea*, *Synchytrium endobioticum* and *Verticillium* species among others. However, the permissible levels for these pathogens range from 0.1 – 20% for G1 – G8 classes, respectively (DAFF 2010). This assessment is based on appearance of symptoms meaning that latent infections can pass the certification process unnoticed. The role of seed borne disease transmission between countries is also supported by Tsror et al. (1999) who demonstrated that importation of seed from northern Europe was the main source of potato pathogen infestation in Israel.

The cultivar effect was taken into account in pathogenicity testing as it affects virulence of pathogens (Merida et al. 1994; Fiers et al. 2010). Cultivars possess diverse levels of resistance to different pathogens (Watkinson et al. 2016) and inoculation of all test isolates onto one crop cultivar may affect results. Our findings support this statement since *M. phaseolina* isolates were more virulent on cv. Mondial than on cv. BP1 on which they caused mild symptoms.

Conclusion

The present study has revealed a variety of fungal microflora associated with potato atypical tuber blemishes in South Africa and Zimbabwe. The work provided an insight into the atypical tuber blemishes affecting the potato industry in South Africa and Zimbabwe. The pathogenicity of BNR AG A, *M. phaseolina* and *R. solani* AG 3-PT in causing tuber defects, charcoal rot and black scurf associated with cracking and elephant hide, respectively was confirmed. Charcoal rot and tuber defects were diagnosed from South African tubers while tubers with corky cracks and elephant hide came from both countries. This is the first report of BNR AG A and *M. phaseolina* causing tuber defects and charcoal rot, respectively in South Africa. This is also the first report of tuber cracks and elephant hide caused by *R. solani* AG 3-PT in Zimbabwe. The results will enable farmers and extension officers to formulate control strategies against these plant diseases. However, the study was not exhaustive. The role played by bacteria, viruses and nematodes in addition to environmental influences in causing atypical tuber blemishes still needs to be investigated.

Acknowledgements

The authors would like to thank the Potato Pathology group at the University of Pretoria for their participation in the collection of samples and maintenance of greenhouse trials. Special thanks go to all stakeholders who provided blemished tubers used in this study. We acknowledge the intellectual and material contribution of The Organisation for Women in Science for the Developing World (OWSD) and the Swedish International Development Agency (SIDA). The National Research Foundation (NRF) is acknowledged for supporting this study and purchasing the DNA sequencing instrument (grant UID: 78566) used at the University of Pretoria.

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