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FIELD TOLERANCE OF SELECTED VARIETIES TO AND FUNGICIDE EFFICACY AGAINST ALTERNARIA BLIGHT OF SWEET POTATO

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ABSTRACT

Alternaria blight (AB) of sweet potato (*Ipomoea batatas* L.), caused by *Alternaria* spp., was recently reported in South Africa, but is common in southern and eastern Africa. Elsewhere in the world, AB is controlled primarily using resistant varieties. Twenty-five sweet potato varieties/breeding lines, from different origins were assessed for tolerance to AB. The materials were planted in fields having a history of AB disease and rated for tolerance based on a General Disease Index (GDI), with the lowest scores representing tolerance, and the higher scores representing susceptibility. Variety 199062-1 had the lowest GDI value, and was the most tolerant to AB; while W119 had the highest GDI value and was the most susceptible to the disease. Other varieties/breeding lines showed a variation in GDI values between most tolerant and most susceptible. Among the fungicides tested under field conditions, the mixture azoxystrobin-difenoconazole was the most effective in reducing AB intensity. Fungicides pyraclostrobin-boscalid, unizeb, azoxystrobin-chlorothalonil and cymoxanil-mancozeb were also effective against the disease.

Key Words: *Alternaria bataticola*, General Disease Index, *Ipomoea batatas*

RÉSUMÉ

L'alternariose (AB) de la patate douce (*Ipomoea batatas* L.), maladie causée par *Alternaria* spp., est une maladie nouvellement rencontrée en Afrique du Sud, mais très fréquemment rencontrée dans les pays au Sud et à l'Est de l'Afrique. Ailleurs dans le monde, l'utilisation de variété résistantes est la première mesure de lutte contre les dégâts causés par AB. Vingt cinq variétés de patate douce de diverses origines ont été évaluées pour leur sensibilité à l'alternariose. Le matériel génétique avait été planté dans des champs ayant une fois infectés de AB et classés tolérants en fonction de leur index général de la maladie (GDI). Les plus petits scores indiquent la tolerance, tandis que les scores les plus élevés indiquent la susceptibilité. La variété 199062-1 avait la plus petite de GDI et donc était le plus tolerant à AB, tandis que la variété W119 présentait la valeur de GDI la plus élevée; par conséquent était la plus susceptible de toutes les variétés. Les autres variétés étaient différentes en ce qui concerne leur GDI, les valeurs de GDI variaient du plus petit au plus grand. Parmi les fongicides testés, le mélange azoxystrobin-difenoconazole était le plus efficace. Les fongicides pyraclostrobin-boscalid, unizeb, azoxystrobin-chlorothalonil et cymoxanil-mancozeb étaient aussi efficace contre l' alternariose.

Mots Clés: Alternaria bataticola, index général de la maladie, *Ipomoea batatas*

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) is the sixth most important food crop in the world, with 105 metric tonnes produced annually. Of this, 95% is grown in developing countries (FAOSTAT, 2009). Orange-fleshed sweet potato varieties are particularly important for having a high beta-carotene content, which helps to alleviate vitamin A deficiency in children, especially in rural areas of Kenya (Lopes and Boiteux, 1994; Mwanga and Ssemakula, 2001).

Alternaria blight (AB) is a serious disease of sweet potato in Central, Eastern and Southern Africa (Van Bruggen, 1984; Anginyah *et al.*, 2001; Kapinga and Carey, 2003; Osiru *et al.*, 2007). In Uganda, AB is ranked the most important constraint to sweet potato production (Anginyah *et al.*, 2001). AB was first reported from South Africa by Narayanin *et al.* (2010a) and has a localised distribution (Thompson *et al.*, 2011). *Alternaria bataticola* Ikata ex W. Yamamoto has been observed to be the main causal pathogen (Lopes and Boiteux, 1994; Osiru *et al.*, 2008) while *A. alternata* Fries (Anginyah *et al.*, 2001) and *A. capsici-annui* Savul. and Sandu (Sivaprakasam *et al.*,1977) also induce AB symptoms on sweet potato (Anginyah *et al.*, 2001).

Since the disease is favoured by wet, humid conditions (Ames *et al.*, 1997; Osiru *et al.*, 2009), and median temperatures of 28°C (Osiru *et al.*, 2007), it has the potential to develop in certain sweet potato production areas of South Africa, and should, therefore, be considered a potential threat to the sweet potato industry in the country.

In most countries, control of AB of sweet potato is limited, with the disease being managed primarily by planting resistant varieties (Anginyah *et al.*, 2001; Mwanga *et al.*, 2003; Osiru *et al.*, 2009). In South Africa, a preliminary glasshouse study showed variation in *Alternaria* blight levels among sweet potato varieties (Narayanin *et al.*, 2010b). Use of pyrimethanil, cyprodinil, fludioxonil, azoxystrobin, difenoconazole and potassium bicarbonate as control measures has been used with success in the USA (Olson *et al.*, 2012). The objective of this study was to evaluate a wide range of sweet potato varieties/breeding lines from different origins under field conditions for tolerance to AB, and screening for efficacy of selected fungicides against AB.

MATERIALS AND METHODS

Study locations. The study was conducted from 2010-2012, at two sites, namely Roodeplaat experimental farm of the Agricultural Research Council, Vegetable and Ornamental Plant Institute (ARC-VOPI) (latitude 25,60398; longitude 28,35429; altitude 1168 m), Pretoria, Gauteng, South Africa, and at the Owen Sithole College of Agriculture (OSCA), Empangeni (latitude 28,72496; longitude 31,89813; altitude 105 m), KwaZulu-Natal, South Africa. At ARC-VOPI only one variety/breeding line was assessed (2010-2011 season) as very low disease intensity in the second growing season (2011-2012) made the disease assessment not possible. On the other hand, the fungicide study was conducted at Bronkhorstspruit, Gauteng, South Africa and ARC-VOPI from 2012-2014.

Variety/breeding line field trials. Disease-free cuttings of 25 sweet potato varieties/breeding lines, of different origin; were manually planted. A 9 m long ridge (plot) was used, with between-ridge spacing of 1 m, and in-ridge spacing of 30 cm. Each plot was replicated three times, in a randomised complete block design. The between-plot ridges and border ridges were planted with a mixture of varieties A15 and Excel, both reported to be susceptible to AB under greenhouse conditions (Narayanin *et al.*, 2010b). The study at both ARC-VOPI and OSCA was conducted in fields having a history of AB disease. Plants received overhead irrigation for two hours in the evening (18.00 - 20.00 hr), twice a week, starting three months after planting to induce optimal conditions for development of AB.

Fields were fertilised with NPK of 18.5:0:18.5 at 500 kg ha^{-1}, applied one day before planting; while limestone ammonium nitrate (LAN, 28%) was applied as a top-dressing three weeks later at 120 kg ha^{-1}. Weeds were removed manually using hand-hoes. Owing to seasonal differences of the localities, experiments were conducted from December to April and February to June at ARC-VOPI and OSCA, respectively.

Fungicide field evaluation. A randomised complete block design, with three replicates, was used. Disease-free cuttings of variety W 119, which is susceptible to AB (Narayanin *et al*., 2010b), were manually planted. Each ridge (representing a fungicide treatment plot) was delimited by two border ridges. Details concerning all fungicides are shown in Table 1. As mentioned above, the ARC-VOPI field trial site had a history of AB; while the Bronkhorstspruit field trial site was a new field. Accordingly, plants at Bronkhorstspruit were artificially inoculated in the evenings, by placing AB diseased leaves on top of the canopy (three infected leaves per spot) at three equally distant points (approx. 2.30 m from each other) per ridge, and overhead irrigated in the evening(16:00-18:00 hr), twice a week to promote natural *Alternaria* infection. Fertiliser application and field maintenance were similar to those used for the variety field trials.

Fungicides were applied when AB symptoms first appeared, and this was repeated every 10 days using a knapsack sprayer. The untreated control plants received only sterile distilled water. Fungicides were applied four and three times at the ARC-VOPI and the Bronkhorstspruit trials, respectively. Plants were not watered for 24 hr, following each fungicide application to reduce wash-off.

Disease assessment. Alternaria infection was too low during the first three months of the growing seasons, to assess the area under the disease progress curve, which entails repeated disease assessments (Jeger and Viljanen-Rollinson, 2001). Thus, disease assessment was conducted once at the end of the growing season when disease levels were highest.

For each plot, three equally distant (approx. 2.3 m from each other) sampling points were taken, with the two outer sampling points located one metre from both ends. In the variety study, both leaves and stems were assessed; while in the fungicide trial plots, only leaves were assessed as AB symptoms developed poorly on the stems.

An open, wooden frame (47 cm x 47 cm) was used to assess leaf disease severity. The frame was placed on top of the canopy at each sampling point. Fully developed leaves inside the frame were rated using the Ordinal Disease Rating scale of 0-4 based on area of leaf necrosis; where 0 = no disease; 1 = <25% covered by small spots (~1mm diameter, no yellowing, no abaxial veinal necrosis; 2 = 25-50% covered by larger spots (~2-3 mm diameter), no yellowing, mild veinal necrosis; 3 = >50% covered, extensive smaller

TABLE 1. Fungicides used in field trials to test for efficacy against *Alternaria* blight of sweet potato at two locations in South Africa

Active ingredients	Dosage	Type of fungicide
Azoxystrobin-chlorothalonil	1.25 ml 100 ml^{-1}	Systemic/preventive
Azoxystrobin-difenoconazole	375 ml 100 ml^{-1}	Systemic/contact
Pyraclostrobin-boscalid	300 g 100 l^{-1}	Systemic
Cymoxanil-mancozeb	3 kg 100 l^{-1}	Preventive
Copper hydroxide	350 ml 100 ml^{-1}	Preventive
Copper hydro. Equiv[a]	150 ml 100 l^{-1}	Preventive
Potassium phosphate	2 l 100 l^{-1}	Systemic
Maneb-zinc oxide	320 ml 100 ml^{-1}	Preventive
Mancozeb	200 g 100 l^{-1}	Preventive
Tap water		

[a]Copper hydro. Equiv = Copper hydroxide equivalent

spots; or a few large spots with a target pattern, extensive veinal necrosis, partial leaf yellowing; 4 = complete yellowing, necrotic, leaves dying and falling off.

For each variety, nine sampling points were taken, with ~225 individual leaves assessed and a total of ~5625 individual leaves assessed for all varieties per field trial. At both fungicide study sites, twenty individual leaves were assessed per sampling point, with 1200 individual leaves assessed for the entire experiment.

A wooden frame of 47 cm x 47 cm, subdivided into nine equal sub-squares with taut wire (0.7 mm diameter), was used to assess stem disease severity. The frame was placed at the same sampling points used to assess leaf diseases. The same ordinal rating scale described above was used to rate the percentage of infected stems. A single, ordinal rating was given for all the stems within each sub-square. For each variety, 81 sub-squares were assessed, with a total of ~2025 sub-squares assessed for all varieties per field trial.

Statistical analysis. The stem and leaf disease ratings were subjected to a General Linear Model (GLM) technique, with a logistic link function. The maximum likelihood estimators (XBeta's) were calculated as index values on an underlying scale (McCullagh and Nelder, 1989). These estimators (location values) were on an interval scale, from negative to positive values, and were added together to produce the General Disease Index (GDI) means, whose values expressed the level of susceptibility/tolerance of varieties/breeding lines to AB. Ranking of varieties and breeding lines was based on GDI values, with the lowest being the most tolerant, and the highest the most susceptible. The variance of each experiment was tested for comparable magnitude, using Levene's test (Levene, 1960), before the analysis of combined data was performed. There was a strong evidence for homogeneity of variances between localities (Table 4); therefore, a weighted analysis of combined data was performed (John and Quenouille, 1977). Fisher's t-Least Significant Difference (LSD) was calculated to compare means of significant effects at 5% significance level (Snedecor and Cochran, 1967). Analysis was performed using SAS/Stat Version 9.2 Statistical Software (SAS, 1999).

RESULTS

Field study. High AB disease levels were naturally present at both field trial sites at the end of both growing seasons (Table 3). GDI values for the different varieties/breeding lines varied continuously from -7.229 to 4.555. Three groups emerged based on GDI values *viz.* tolerant, intermediate and susceptible. 199062-1 was the most tolerant variety/breeding line (GDI-7.229), followed by Muvhelo, 2007-3-10 and Impilo. W119 was the most susceptible variety (GDI 4.555) (Table 3). Varieties/breeding lines with intermediate tolerance included Bophelo, 2004-5-2, Purple Sunset, 2002-8-2, Monate, Ndou, 2007-1-3 and 2007-2-12.

In addition, there were significant differences in disease levels between localities and between growing seasons (Table 4). During the 2010-2011 growing season, the OSCA field trial recorded a significantly higher disease severity than ARC-VOPI field trial. In the 2011-2012 growing season, the infection level was very low at ARC-VOPI field trial and data could not be analysed statistically. The disease severity was significantly higher at OSCA field trial in 2011 than in 2012 (Table 4).

Fungicide study. Table 5 shows the performance of selected fungicides against AB. The first symptoms of AB appeared 2 months after planting. Results from the three field trials showed that azoxystrobin-difenoconazole was the most effective fungicide treatment. Pyraclostrobin-boscalid, mancozeb, azoxystrobin-chlorothalonil and cymoxanil-mancozebshowed intermediate fungicide efficacy, while copper-hydroxide-copper equivalent was the least effective.

DISCUSSION

The Owen Sithole College of Agriculture trial site experienced a mean maximum relative humidity of 95% and the optimum temperature of 28°C in both seasons (Table 2). It is likely that these conditions were suitable to the spread of AB as

TABLE 2. Temperature, rainfall and relative humidity values at ARC-VOPI[a] and OSCA[b] from December to April 2010-2012 and February to June 2011-2012, respectively

Parameter	ARC-VOPI[a]		OSCA[b]	
	2010/2011	2011/2012	2011	2012
Mean maximum temperature (°C)	28	29	28	28
Mean minimum temperature (°C)	15	15	17	17
Total rainfall (mm)	888	420	277	155
Mean maximum relative humidity (%)	91	89	95	95
Mean minimum relative humidity (%)	39	32	48	49

[a] ARC-VOPI - Agricultural Research Council - Vegetable and Ornamental Plant Institute, Pretoria, Gauteng, South Africa
[b] OSCA -Owen Sithole College of Agriculture, Empangeni, KwaZulu-Natal, South Africa

TABLE 3. Sweet potato variety/breeding lines assessed for *Alternaria* blight disease severity (2010-2012), expressed as General Disease Index means using an interval scale

Variety/ breeding line	Origin	Type	Tuber colour	GDI[a] and t-grouping
W119	USA	Variety	Orange	4.555 a
2004-3-9	ARC-VOPI[b]	Line	Orange	4.342 a
2004-9-1	ARC-VOPI	Line	Orange	4.316 a
Excel/A15	USA[c]/UKZN[d]	Varieties	Orange/Orange	4.173 a
Resisto	USA	Variety	Orange	4.155 a
Isondlo	ARC-VOPI	Variety	Orange	4.028 a
Hernandez	USA	Variety	Orange	3.88 a
2004-16-1	ARC-VOPI	Line	Orange	3.504 a
Beauregard	USA	Variety	Orange	3.489 a
2004-3-1	ARC-VOPI	Line	Orange	3.232 ab
Blesbok	ARC-VOPI	Variety	Cream	3.174 ab
2004-9-2	ARC-VOPI	Line	Orange	2.96 ab
2003-23-6	ARC-VOPI	Line	Orange	2.849 ab
Bophelo	ARC-VOPI	Variety	Orange	-0.502 cd
2004-5-2	ARC-VOPI	Line	Orange	-0.665 cd
Purple sunset	ARC-VOPI	Variety	Orange	-0.805 cde
2002-8-2	ARC-VOPI	Line	Orange	-1.521 cde
Monate	ARC-VOPI	Variety	Cream	-1.6 cde
Ndou	ARC-VOPI	Variety	Cream	-1.781 cde
2007-1-3	Uganda	Line	Orange	-2.707 cde
2007-2-12	Uganda	Line	Orange	-3.034 cde
Impilo	ARC-VOPI	Variety	Orange	-3.3 def
2007-3-10	Uganda	Line	Orange	-4.087 def
Muvhelo	ARC-VOPI	Variety	Cream	-6.553 def
199062-1	CIP[e]	Variety	Orange	-7.229 def
LSD (P = 0.05)				3.35

t-Grouping = Values followed by the same letter do not differ significantly according to Student's t-Least significant difference test (P<0.05)
[a]GDI - General Disease Index; [b]ARC-VOPI - Agricultural Research Council-Vegetable and Ornamental Plant Institute, Pretoria, South Africa; [c]USA - United States of America; [d]UKZN - University of KwaZulu-Natal, Kwazulu-Natal, South Africa; [e]CIP - International Potato Centre; LSD – Least Significant Difference

this site recorded higher *Alternaria* infection than the ARC-VOPI trial site. Rotem *et al.* (1989) showed the role of high humidity in inducing *Alternaria* infection. Similarly, Pleysier *et al.* (2006) reported that humidity and temperature significantly influenced the infection of *A. alternata* on *Paulownia fortunei* (Seem.) Hemsl. However, the disease did not develop well at ARC-VOPI in the second growing (2011-2012). Several factors can lead to a decrease in disease severity at a given site including humidity, temperature, rainfall, sowing date or pathogen virulence (Kumar and Srivastava, 2013).

TABLE 4. *Alternaria* blight of sweet potato disease severity expressed as General Disease Index (GDI) means on an interval scale at two locations

Location	GDI	t-Grouping
OSCA[a] 2011	-2.696	b
ARC-VOPI[b] 2010/2011	1.323	a
OSCA 2012	1.807	a
ARC-VOPI 2011/2012	-	-
LSD (P = 0.05)	1.094	

t-Grouping = Values followed by the same letter do not differ significantly according to Student's t-least significant test (P <0.05)
[a]OSCA = Owen Sithole College of Agriculture, Empangeni, Kwazulu-Natal, South Africa
[b]ARC - VOPI = Agricultural Research Council, Vegetable and Ornamental Plant Institute, Pretoria, South Africa

The results of this study showed the reaction of sweet potato varieties and breeding lines during two growing seasons in different localities. The GDI ranking of varieties against AB showed a wide range of tolerance. The variety 199062-1 was the most tolerant plant material against the disease. 199062-1 originates from Peru and has been also reported tolerant to AB in various sweet potato growing countries (Sunette Laurie, personal communication). Variety rankings suggested that Muvhelo, Monate, Ndou and Impilo were tolerant to AB under field conditions. A similar study conducted by Narayanin *et al.* (2010b) reported these three varieties as being tolerant to AB under glasshouse conditions. On the contrary, Beauregard, Hernandez, Isondlo, Resisto and Excel showed susceptibility to AB while W119 was the most susceptable variety. These findings are in agreement with Narayanin *et al.* (2010b) who observed that W119, Excel, Beauregard and Resisto were susceptible to AB under glasshouse conditions. W119 and Resisto are originally from the USA (Kapinga *et al.*, 2010) and are currently cultivated in many African countries including Mozambique, Madagascar and South Africa (Kapinga *et al.*, 2010).

The breeding lines 2007-3-10, 2007-2-12 and 2007-1-3 were tolerant to AB. Little has been

TABLE 5. Field reaction of nine fungicides against *Alternaria* blight of sweet potato

Fungicides	ARC-VOPI[a]	Bronkhorstspruit	ARC-VOPI
	2012-2013	2012-2013	2013-2014
Azoxystrobin-difenoconazole	0.89	0.39d	0.84
Pyraclostrobin-boscalid	1.16	0.54	0.93
Mancozeb	0.94	0.57	1.26
Azoxystrobin-chlorothalonil	1.33	0.68	0.96
Cymoxanil-mancozeb	1.36	0.61	1.06
Maneb-zinc oxide	1.14	1.22	1.64
Potassium phosphate	1.29	1.61	1.64
Copper hydroxide	2.03	1.22	1.71
Copper hydroxide-copper equivalent	2.48	2.34	1.96
Control	2.82	2.87	2.74
LSD (0.05)	0.478	0.44	0.38

[a]ARC - VOPI = Agricultural Research Council, Vegetable and Ornamental Plant Institute, Pretoria, South Africa

published on the reaction of these breeding lines against AB. Moreover, these tolerant materials represent reliable sources of resistance, which can be used in future breeding programs to develop tolerant varieties against AB. Other breeding lines such as 2004-3-1, 2004-16-1 and 2004-9-1 exhibited susceptibility to AB with 2004-3-9 being the most susceptible. Results from the breeding lines showed that AB disease was not tuber colour-dependent as previously suggested by Van Bruggen (1984) and Du Plooy *et al.* (2008) who reported that sweet potato with red tubers were more susceptible to *Alternaria* infection than cream coloured varieties. In this study all tolerant and susceptible varieties/breeding line had the orange tuber colour.

In the fungicide study the mixture azoxystrobin-difenoconazole was the most effective in controlling AB. Both azoxystrobin and difenoconazole have been reported to act as protectant and curative fungicides, respectively against *Alternaria solani* (Ellis & Martin) Sorauer (Horsfield *et al.*, 2010). The results of this experiment agrees with research conducted by Reuveni *et al.* (2002) on *A. solani* and *A. alternata* on red deciduous apple which showed that azoxystrobin and difenoconazole were both effective in controlling these two pathogens. A spraying program that incorporates both protectant and curative active ingredients has proved to be effective in reducing plant diseases. The results from this work showed that at both ARC-VOPI and Bronkhorstspruit, plants sprayed with a mixture of pyraclostrobin-boscalid recorded low disease. This finding was echoed earlier by Pasche and Gudmestad (2008) and Gudmestad *et al.* (2013) in *A. solani*. However, the continuous use of pyrachlostrobin and boscalid can lead to the rapid development of resistance strains of *Alternaria* as these compounds have a site-specific mode of action (Avenot and Michailides, 2007; Avenot *et al.*, 2008). In Australia, farmers are discouraged from applying strobilurin fungicides, to which pyrachlostrobin belongs, consecutively and their use should never exceed four applications per annum (Infopest, 2003).

Data from this study revealed that the mixture cymoxanil-mancozeb was also effective against AB because plants treated with these compounds recorded low disease intensity. Similarly, Catão *et al.* (2013) also reported this mixture to be effective against *A. tomatopila* Simmons. Mancozeb is often used in combination with other active ingredients as it has many sites of action, therefore delaying the occurrence of pathogen resistant strains (Gullino *et al.*, 2010). Copper hydroxide and copper hydroxide-copper equivalent were the least effective compounds in controlling AB among all the fungicides tested during all the growing seasons and at all sites. Similar results were echoed by Raid and Timmer (1999) on *A. alternata*. To our knowledge currently there is no registered fungicide for *Alternaria* diseases of sweet potato in South (or southern) Africa. These results provide information for chemical companies indicating the most promising active ingredients that control AB under field conditions.

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