Effects of wetness duration, inoculum concentration and temperature on the development of *Alternaria* blight on transplants of sweet potato

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Abstract

***Alternaria* blight (AB) of sweet potato caused by *Alternaria bataticola* is a limiting factor for sweet potato industries in various countries. The disease has been recently reported in South Africa. Thorough literature search indicates that factors influencing the development of AB have not been studied adequately. In the current study, wetness duration, inoculum concentration and temperature/wetness duration interaction were evaluated for their effects on the disease incidence and disease severity of AB of sweet potato. Cultivars W119 reported susceptible to AB were inoculated with a spore suspension of *A. bataticola* (PPRI 11930) under glasshouse and plant growth chamber conditions. In the wetness duration experiment at the constant temperature of 28°C each increase of wetness duration significantly increased the mean disease incidence (MDI) and mean disease severity (MDS). Exposure for 48h was the optimum condition of this experiment, as it resulted in the highest MDI and MDS. In the inoculum concentration experiment the MDI and MDS increased significantly as inoculum concentration increased but there was no significant difference between $3x10^4$ conidia mL^{-1} and the highest inoculum concentration of $5x10^4$ conidia mL^{-1}. In the interaction of temperature and wetness duration the optimal temperatures and wetness duration in this study were 20 and 25°C for 48 h, respectively. Exposure at 35°C irrespective of wetness duration period did not produce any AB symptoms as shown by the MDI and MDS.**

Keywords: *Alternaria bataticola*, disease assessment, disease incidence, disease severity, *Ipomoea batatas*

INTRODUCTION

A devastating fungal disease named *Alternaria* blight (AB) represents one of the major constraints in sweet potato (*Ipomoea batatas* [L.] Lam.) cultivation in some agro-ecological areas (Lopes and Boiteux, 1994; Anginyah et al., 2001; Mwanga et al., 2003; Osiru et al., 2007). The disease has been reported in different African countries including Ethiopia (Van Bruggen, 1984), Burundi (Simbashizweko and Perreaux, 1988), Kenya (Anginyah et al., 2001), Malawi and Zimbabwe (Kapinga and Carey, 2003), Uganda (Osiru et al., 2007) and South Africa (Narayanin et al., 2010a). Elsewhere in the world the disease is also known from India (Sivaprakasam et al., 1977), Brazil (Lopes and Boiteux, 1994), Cuba (González and Martínez, 2008) and the USA (Olson et al., 2012).

More studies on AB have reported *A. bataticola* Ikata ex W. Yamamoto as the causal agent of AB of sweet potato (Lopes and Boiteux, 1994; Anginyah et al., 2001; Mwanga et al., 2003). However, recently several *Alternaria* spp. have been reported to be associated with AB on sweet potato, including the newly reported species *A. ipomoeae* sp. nov. M. Truter, Woudenb. & Crous, and *A. neoipomoeae* sp. nov. M. Truter, Woudenb. & Crous, (Woudenberg et al., 2014).

Despite the devastating effect of AB on sweet potato cultivation little is known about the factors affecting the development of the disease. The objective of this investigation was to evaluate the effect of wetness duration, inoculum concentration and

temperature/wetness duration interaction on the development of AB of sweet potato caused by *A. bataticola* under glasshouse and plant growth chamber conditions.

MATERIALS AND METHODS

Plant materials and preparation of inoculum

A pathogenic isolate of *A. bataticola* (PPRI 11930) was provided by the National Collection of Fungi of the Agricultural Research Council-Plant Protection Research, Pretoria, South Africa. Single spore cultures of *A. bataticola* were sub-cultured in 9 cm diameter Petri dishes on potato carrot agar (PCA) and incubated under 12 h UV light for 14 days at room temperature to induce sporulation. A spore suspension was prepared by flooding the plates with sterile distilled water. The mycelium was gently scraped with a sterile plastic rod to dislodge spores and 0.01% of the surfactant Tween 20 was added. Spore concentration was determined using a haemocytometer.

Disease-free sweet potato cuttings of variety W119 reported to be susceptible to AB (Narayanin et al., 2010b; Kandolo et al., 2016) obtained from the Agricultural Research Council-Vegetable and Ornamental Plant Institute, Pretoria, were planted in 128 cell polystyrene (670 x 340 mm; 60 mm deep) seedling trays (filled with pasteurised loam soil) with two nodes of the cuttings inserted in the soil and two other nodes left above the soil. The trays were maintained in a glasshouse at 28 ± 1°C and watered every 2 days until runoff for 2 weeks. Seedlings were then transplanted into pots (15 cm diameter; 12 cm deep) filled with pasteurised soil (Braaks, Pretoria) and maintained at 28 ± 1°C for 2 weeks before being used in subsequent experiments. Each pot contained one transplant. The transplants were fertilized with Multifeed foliar feeding and fertilization (Plaaskem (Pty) Ltd, South Africa) according to manufacturer's instructions.

In all experiments, except in the inoculum concentration experiment, 4-week old transplants were inoculated in the evening with a spore suspension of *A. bataticola* at $3x10^4$ conidia mL^{-1} using a hand spray bottle until runoff. Inoculated transplants were subject to 0, 12, 24 and 48 h wetness duration (i.e transparent polythene bags wetted inside with sterile tap water that covered the pots and held in place with rubber bands) (Figure 1).

Immediately after wetness duration polythene bags were removed and transplants were maintained in the glasshouse at 28 ± 1°C except for the temperature/wetness duration experiment.

To determine the effect of inoculum concentration the transplants were inoculated with spore concentrations of $5x10^3$; $1x10^4$; $3x10^4$ or $5x10^4$ conidia mL^{-1} until runoff. Inoculated transplants were subjected to 48 h wetness duration as described above.

In the temperature/wetness duration interaction experiment inoculated transplants were placed inside the plant growth chamber (Controlled Environments, Ltd Model POW36, 7L 13515 Winnipeg Manitoba, Canada) set at 20, 25, 30 or 35°C as main plot factor. Within the plant growth chamber inoculated transplants were subjected to 0, 12, 24 and 48 h wetness duration. The temperature in the growth chambers was set 24 h before the start of the experiment and deviated by ± 1°C throughout the experiment. All control transplants were sprayed with sterile distilled water mixed with 0.01% of Tween 20.

In all experiments, pots were arranged in a Randomised Complete Block Design (RCBD) with five block replicates. Each block replicate consisted of six pots containing one transplant per pot replication. All the experiments were repeated twice.

Disease assessment

Leaves were assessed for disease severity and disease incidence seven days after inoculation using visual observations. The disease severity was measured using the following ordinal disease rating scale based on area of leaf necrosis: 0 = no disease; 1 = < 25%; 2 = 25-50%; 3 = > 50% and 4 = dead leaf (or leaf that turned yellow). The disease incidence was calculated by counting the number of infected leaves x 100 divided by the total number of leaves per transplant.

Data analysis

The mean disease incidence (MDI) and mean disease severity (MDS) measured from the above experiments were subjected to combined analysis of variance (ANOVA) for the

two repeats after confirming that the variances of each repeat was of comparable magnitude using Levene's test for homogeneity (Levene, 1960; John and Quenouille, 1977). The standardized residuals were tested for deviations from normality using Shapiro-Wilk's test (Shapiro and Wilk, 1965). In cases where significant deviation from normality was evident and the deviation was due to skewness, outliers were removed until data was of normal or symmetric distribution (Glass et al., 1972). Student's *t*-least significant difference (LSD) was calculated at 5% significance level to compare means of significant source effects. Data analysis was performed with SAS version 9.3 statistical software (SAS Institute, 1999).

Figure 1. Sweet potato transplants inoculated with *A. bataticola* and covered with transparent polythene bags.

RESULTS

Wetness duration

In the wetness duration experiment at the constant temperature of 28°C each increase of wetness duration significantly increased the MDI and MDS. Exposure at 48 h was the optimum condition of this experiment, as it resulted in MDI and MDS of 81.7 and 2.4, respectively (Table 1). Furthermore, there was no significant difference between 0 h and the untreated control as their MDI and MDS were statistically similar. The disease did not develop on the untreated control transplants (Table 1).

Table 1. Effect of wetness duration on the development of *Alternaria* blight of sweet potato grown at 28°C

	Mean disease	
Treatment	Incidence (%)	Severity (%)
48 h	81.7a	2.4a
24 h	76.8b	1.54b
12 h	66.1c	1.1c
0 h	1.1d	0.01d
Control	0.0d	0.0d
LSD (p=0.05)	4.8	0.21

Means within columns with the same letter do not differ significantly according to Student's *t*-Least significant difference (LSD) test (P<0.005).

Inoculum concentration

In the inoculum concentration experiment the MDI and MDS increased significantly as inoculum concentration increased. However, there was no significant difference between $3x10^4$ conidia mL^{-1} and the highest inoculum concentration of $5x10^4$ conidia mL^{-1} as their MDI and MDS were (89,7; 2.5) and (88.4; 2.6) respectively (Table 2).

Interaction temperature and wetness duration

At 20, 25 and 30°C the MDI and MDS increased as wetness duration increased. The MDI and MDS were significantly higher at the above temperatures when transplants were exposed at 48 h wetness duration. The optimal temperatures and wetness duration in experiments were 20 and 25°C for 48 h, as shown by the highest MDI (88.1 and 88.0) and MDS (1.2 and 2.1), respectively. There was a decline of MDI (71.2) and MDS (1.0) when inoculated transplants were exposed at 30°C for 48 h wetness duration (Table 3). However, exposure at 35°C irrespective of wetness duration period did not produce any AB symptoms as shown by the MDI and MDS. We can conclude that 35°C was detrimental to the development of *A. bataticola* in the current study. In the absence of wetness duration (0 h) the MDI and MDS were statistically similar to the untreated control at all ranges of temperatures. There were no symptoms of AB in the untreated control (Table 3).

Table 2. Effect of inoculum concentrations on the development of *Alternaria* blight of sweet potato grown at 28 °C

Conidia mL^{-1}	Mean disease incidence (%)	Mean disease severity (%)
$5x10^4$	88.4a	2.6a
$3x10^4$	89.7a	2.5a
$1x10^4$	76.0b	1.5b
$5x10^3$	38.0c	0.4c
Control	0.0d	0.0d
LSD (p=0.05)	7.2	0.2

Means within columns with the same letter do not differ significantly according to Student's *t*-Least significant difference (LSD) test (P<0.005).

Table 3. Temperature/wetness duration interaction on the development of *Alternaria* blight of sweet potato, inoculated with *Alternaria bataticola* at $3x10^4$ conidia mL-1.

	MDI[a] (%)				MDS[b] (%)			
	Temp[c]				Temp			
Wet. Durat[d]	20°C	25°C	30°C	35°C	20°C	25°C	30°C	35°C
48 h	88.1	88a	71.2a	0a	1.2a	2.1a	1.0a	0a
24 h	53b	81.4a	56b	0a	0.5b	1.3b	0.6b	0a
12 h	12.7c	38.4b	47.4b	0a	0.1c	0.4c	0.5b	0a
0 h	1.4d	0.0c	2.3c	0a	0.0c	0.0d	0.02c	0a
Control	0.0d	0.0c	0.0a	0a	0.0c	0.0d	0.0c	0a
LSD (p=0.05)	12.6	11.1	10.7		0.17	0.3	0.14	

Means within columns with the same letter do not differ significantly according to Student's *t*-Least significant difference (LSD) test (P<0.005); [a]MDI = mean disease incidence; [b]MDS = mean disease severity; [c]Temp = temperature; [d]Wet. Durat = wetness duration.

DISCUSSION

Results from this study show the importance of temperature, humidity and inoculum concentration in the development of AB caused by *A. bataticola* on sweet potato transplants. The constant temperature of 28°C and 48 h wetness duration were the most conducive conditions for establishment of AB. Similar results were obtained for *A. brassicae* (Berk.) Sacc. and *A. solani* Sorauer on oilseed rape (*Brassica napus* L.) (Hong and Fitt, 1995) and tomato (*Solanum lycopersicum* L.) (Vloutoglou and Kalogerakis, 2000), respectively.

The increase of inoculum concentration resulted in an increase of AB. The disease was particularly severe in both $3x10^4$ and $5x10^4$ conidial mL-1. These results are in line with

those of Coffey et al. (1975) and Vloutoglou and Kalogerakis (2000) on early blight of tomato caused by *A. solani*. Similarly, Southwell et al. (1980) and Hong and Fitt (1995) demonstrated a positive correlation between increased inoculum density and increased infection by *A. alternata* (Fr.) Keissl. and *A. brassicae* on durum wheat (*Triticum durum* Desf.) and oilseed rape, respectively. Our study showed that an increase from $3x10^4$ to $5x10^4$ conidia mL^{-1} did not increase AB. This observation was also made earlier for *A. linicola* Groves and Skolko (Vloutoglou et al., 1999) and *Spilocaea oleagina* Castagne (Hughes) (Obanor et al., 2011). Rotem (1994) explained that at high inoculum concentrations the spores of some *Alternaria* species may clump together, which results in self-inhibition of germination.

In the temperature/wetness duration interaction AB was severe when inoculated transplants were exposed at 20 and 25°C for 48 h. Similarly, in other studies *A. carthami* Chowdhury (McRae et al., 1984), *A. cirsinoxia* Simmons and Mortensen (Green and Bailey, 2000) and *A. alternata* (Pleysier et al., 2006) induced disease at 20°C and 25°C. In our study there was a decline in AB development when inoculated transplants were exposed to 30°C and 48 h wetness duration compared to 20 and 25 °C. A similar finding was reported by Pleysier et al. (2006) on *A. alternata* on the leaves of *Paulownia fortunei* Hemsl. AB did not develop at 35°C for all wetness durations.

Similarly, 0 h wetness duration did not differ significantly to the untreated control, as the MDI and MDS were statistically the same. We can hypothesize that at 0 h wetness duration there was not sufficient humidity to facilitate the presence of free water on the leaf surface of inoculated sweet potato transplants, which according to Rotem (1994) is a prerequisite condition for the germination and infection of *Alternaria* species.

As AB symptoms are primarily situated on the leaves and the vines (Osiru et al., 2007) it is of paramount importance to ensure that sweet potato transplants and cuttings (vines) supplied to different sweet potato growers are AB-disease free. It is possible for a single infected cutting to initiate a primary infection in the field especially when conditions are conducive to the development of the disease.

CONCLUSION

The ideal conditions for the spread of AB on sweet potato were 48 h wetness duration and the temperatures of 20 and 25°C. However, more parameters and other parameter combinations should be further investigated to elucidate all the factors involved in the development of AB of sweet potato caused by *A. bataticola*.

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