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Distribution of Alternaria leaf blight of sunflowers caused by *Alternaria alternata* in South Africa

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Abstract

Alternaria leaf blight (ALB) has been shown in recent years to be one of the major potential disease threats of sunflower capable of causing yield losses in all major production areas. The aim of this study was to determine the causal agent, prevalence and geographical distribution of ALB in the major sunflower production areas of South Africa. Surveys were conducted during 2012/13, 2013/14 and 2014/15 growing seasons at commercial sunflower production fields and at commercial cultivar trials. In the three growing seasons, twenty-nine sunflower commercial production sites were surveyed for ALB disease severity. Furthermore, four cultivars (AGSUN8251, PHB65A25, SY4200 and PAN7049) were surveyed for ALB during cultivar trials in a total of 25 localities during the three growing seasons. The plants were surveyed between 90 to 120 days after planting and leaves showing ALB symptoms were collected. *Alternaria alternata* was identified as the primary disease-causing organism of ALB in all the fields. The site Bothaville-Wesselsbron consistently had the lowest ALB disease severity during the 2013/14 and 2014/15 growing seasons, whereas Potchefstroom had the highest disease severity in all three growing seasons. Pearson's correlation coefficient was greatest for temperature (r = 0.6 in 2012/13, r = 0.71 in 2013/14 and r = 0.84 in 2014/15) and disease severity in all the growing seasons. Information about the distribution of sunflower diseases is important and this survey demonstrated that *A. alternata* is widespread across sunflower production areas in South Africa and may result in potential yield losses.

Keywords: Cultivar trials, disease severity, environmental factors, geographical distribution, *Helianthus annuus*, survey

1 Introduction

Sunflower (*Helianthus annuus* L.) is an important oilseed crop in South Africa and is used as a source of vegetable oils. The area planted to sunflower seed for commercial use during the 2017 season was 635 750 ha (DAFF, 2017). Sunflower is grown in rotation with maize in some parts of North West, Limpopo, Free State, Mpumalanga and Gauteng provinces.

Studies by Allen *et al.* (1983) and Calvet *et al.* (2005) have shown that Alternaria leaf blight can cause up to 80 % yield loss during sunflower production. Alternaria leaf blight has been recognised as a potentially damaging disease in India, Yugoslavia, Australia, Tanganyika and Uganda (Carson, 1985; Prathuangwong *et al.*, 1991). The main causal agent of Alternaria leaf blight of sunflower is *Alternariaster helianthi* (Hansford) E.G. Simmons (syn. *Alternaria helianthi* (Hansford) Tubaki and Nishihara) (Chattopadhyay, 1999; Prasad *et al.*, 2009). However, in recent studies done in South Africa, *Alternaria alternata* Kiessler was identified as the primary disease-causing organism of ALB (Kgatle *et al.*, 2018).

Alternaria leaf blight symptoms are characterised by circular, brown lesions that are surrounded by a chlorotic halo, which coalesce to form larger lesions as the epidemic pro-

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gresses (Allen *et al*., 1983; Mirza *et al*., 1984). Alternaria leaf blight of sunflower is most common in areas that experience extended periods of wet weather in the summer accompanied by mean and daily temperatures between 25 and 30 °C, and the maximum temperature is around 35 °C (Timmer *et al*., 2003; Prasad *et al*., 2009).

In South Africa, comprehensive information on the distribution and severity of Alternaria leaf blight of sunflower in various agro-ecological zones is not available. Consequently, the aim of this study was to determine the prevalence and geographical distribution of Alternaria leaf blight; to determine the most resistant of four sunflower cultivars to Alternaria leaf blight and to determine the correlation between disease severity and geographical location and geographical conditions in the major sunflower production areas.

2 Materials and methods

2.1 Sampling information

Surveys were conducted at 29 sunflower commercial production sites for Alternaria leaf blight disease severity during three growing seasons (where 10, 10 and 9 localities were surveyed during the 2012/13, 2013/2014 and 2014/15 seasons, respectively) (Fig. 1). Furthermore, cultivar trial surveys were conducted at 25 localities (where 12, 6 and 7 localities were surveyed during the 2012/13, 2013/2014 and 2014/15 seasons, respectively). Cultivar trial surveys were conducted at Agricultural Research Council-Grain Crop Institute (ARC-GCI), Potchefstroom national cultivar trials, seed companies, and sunflower producer farms between 90 to 120 days after planting.

The surveyed localities represented the diverse sunflower growing regions in South Africa. Therefore, the survey varied in the visited localities and number of farms visited annually, however, comparisons among the four cultivars, production regions (which had similar environmental conditions) and number of plants surveyed were kept constant with the intention of surveying as many localities as possible. The trials received the conventional cultural practices of commercial sunflower fields; sowing and top-dressing fertilisation including basal application of 150 kg ha^{-1} blending fertiliser (3N:2P:1K (25) + 0.5 % Zn) incorporated into the seedbed before planting; spraying against post-emerging weeds with the herbicide Euro-Lightning® (BASF, Midrand); and sprinkler irrigation when necessary.

Four cultivars (AGSUN8251, PHB65A25, SYN4200 and PAN7049) were selected for the cultivar trial survey and Alternaria leaf blight disease severity was determined. The cultivar trials were conducted using a randomised block design of four replicates. Furthermore, 30 plants were randomly selected and surveyed on each of the producers fields and disease severity was rated on percentage leaf area loss. The disease severity was based on a scale described in Boedoab *et al*. (2010): where 0 = no disease, 1 = 1 to 25 % leaf infection, 2 = 26 to 50 % leaf infection, 3 = 51 to 75 % leaf infection, 4 = 76 to 100 % leaf infection. Separation of means was done using the least significant difference (LSD) and Fisher's protected test ($p \leq 0.05$). The disease severity was analysed using the standard analysis of variance (ANOVA) using SAS v9.4 (SAS Institute Inc., 2013) statistical package. Pearson's correlations were used to correlate Alternaria blight leaf disease severity and environmental conditions, which were temperature (average temperature of the respective growing seasons, 2012/13, 2013/14 and 2014/15), relative humidity and rainfall of the respective geographical localities received from Annex Table S1.

2.2 Fungal isolation and identification

Sunflower leaves displaying Alternaria leaf blight symptoms were sampled from each surveyed cultivar and sunflower production field for isolation and identification as reported by Kgatle *et al*. (2018). The selected *Alternaria* isolates recovered from the leaf samples were identified based on morphological and molecular characteristics (Kgatle *et al*., 2018).

3 Results

The Alternaria leaf blight symptoms on sunflower that were seen on the foliage were brown, oval to circular spots with a pale margin and yellow halo (Fig. 2). As the infection became more severe, the lesions became irregular by coalescing (encircled area in Fig. 2), leading to blight and premature defoliation and death of the plant. Spots were found mainly on the leaves, but also on other plant parts such as stems, petioles, sepals and petals. Morphological characteristics and multi-gene phylogentic analyses of the 12 selected isolates from symptomatic leaf material (Annex Table S2) (Kgatle *et al*., 2018) indicated that *Alternaria alternata* was the primary disease causing organism of Alternaria leaf blight in the fields sampled.

Surveys showed that Alternaria leaf blight was widespread in all major sunflower production areas. The average Alternaria leaf blight disease severity over the growing seasons was 51.27 %. ANOVA indicated there was a significant difference of Alternaria leaf blight disease severity among the four cultivars within the respective growing seasons ($p \leq 0.05$) but there was however no significant difference overall in the three growing seasons among the four cultivars.

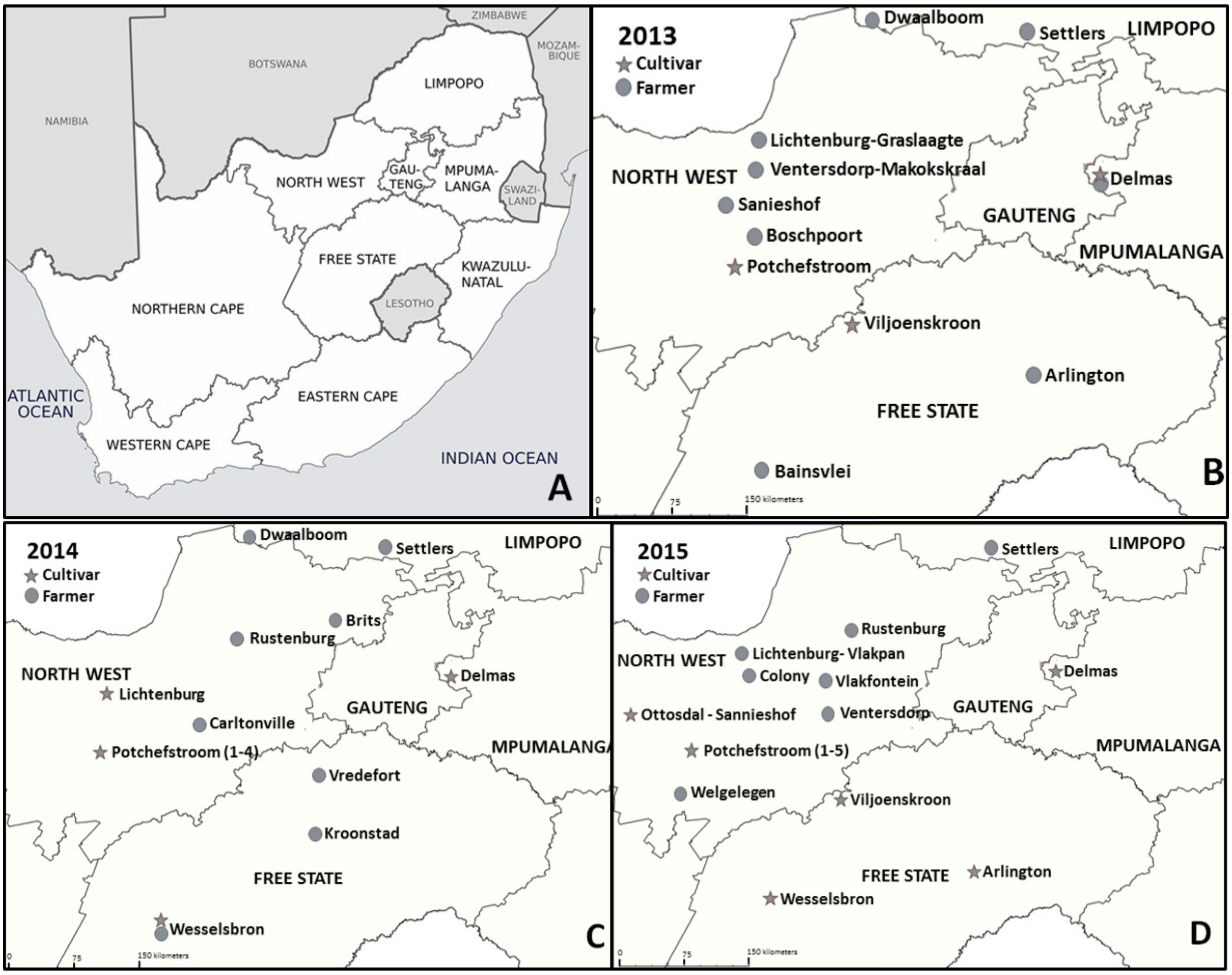

Fig. 1: *(A) Map depicting the provinces of South Africa and its neighbouring countries. Map indicating sunflower cultivar trials and commercial farm localities surveyed in the Free State, North West, Gauteng, Mpumalanga and Limpopo provinces of South Africa during the (B) 2012/13, (C) 2013/14 and (D) 2014/15 production season.*

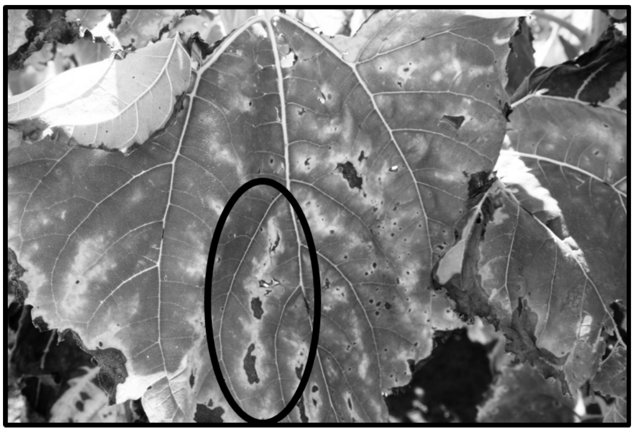

Fig. 2: *Typical Alternaria leaf blight symptoms seen in sunflower growing areas of South Africa. Encircled area indicates coalescence of lesions.*

There were no locality and cultivar interactions noted over the growing seasons. However, there was a significant difference (4.6) between the 2012/13 and 2013/14/15 seasons, which showed a decrease in disease severity during the cultivar trials (Table 1).

Table 2 shows that in the 2012/13 national cultivar trial results, Alternaria leaf blight disease severity on sunflower was significantly higher at Potchefstroom (locality 5) with a mean of 78.2 % (± 11) and lowest at Bainsvlei with a mean of 47.1 % (± 10.3) in the four cultivars. Furthermore, Potchefstroom (localities 2 and 4) cultivar fields had a significantly (6.6 %) higher Alternaria leaf blight severity than Potchefstroom (locality 3), Delmas and Bainsvlei ($p \leq 0.05$).

In the 2013/14 season, Bothaville-Wesselsbron had the lowest Alternaria leaf blight disease severity rating with a mean of 23.1 % (± 11.4), and Potchefstroom (locality 5) the highest 79.1 % (± 12.6) (Table 2). However, some of

Table 1: *The means of the Alternaria leaf blight disease severities surveyed at sunflower production areas during cultivar trials of the 2012/13, 2013/14 and 2014/15 growing seasons.*

	Alternaria leaf blight disease severity (%)			
Sunflower cultivar	*2012/13 season*	*2013/14 season*	*2014/15 season*	*cultivar mean*
AGSUN8251	63.03^{ab}	47.06^{a}	45.11^{b}	51.73^{a}
PAN7049	63.05^{ab}	35.92^{b}	48.00^{b}	48.99^{a}
PHB65A25	64.97^{a}	47.77^{a}	44.50^{b}	52.13^{a}
SYN4200	58.22^{b}	44.75^{a}	52.94^{a}	51.97^{a}
Cultivar mean	62.32^{A}	43.88^{B}	47.64^{B}	51.27
L.S.D.	4.995	6.240	3.586	5.204
CV%	1.9	6.9	0.9	1.3

* For each variable and growing season, means followed by the same lower case letter are not significantly different by Fisher's protected least signifiant difference test ($p \leq 0.05$). Grand means followed by the same upper case letter indicate no significant difference between the three growing seasons, where the Fisher's protected least significant difference test was 4.621 ($p \leq 0.05$) between the three growing seasons (CV % was 9.3).

Table 2: *The means of Alternaria leaf blight disease severities taken from sunflower production areas in South Africa during cultivar trials of the 2012/13, 2013/14 and 2014/15 season.*

	Alternaria leaf blight severity (%)		
Locality	*season 2012/13*	*2013/14*	*2014/15*
Delmas	58.7^{c}	42.7^{c}	55^{cd}
Potchefstroom 2	67.3^{b}	28.7^{d}	39.6^{ef}
Potchefstroom 3	56.1^{cd}	27.6^{d}	55.6^{cd}
Potchefstroom 5	78.2^{a}	79.1^{a}	76.8^{a}
Potchefstroom 4	67.2^{b}	-	59.8^{c}
Potchefstroom 1 - Agricol	61.7^{bc}	-	39.5^{ef}
Bainsvlei	47.1^{d}	-	38.1^{f}
Bothaville - Wesselsbron	-	23.3^{d}	17.7^{g}
Viljoenskroon	-	-	52.3^{d}
Kroonstad	-	-	69.4^{b}
Ottosdal - Sannieshof	-	-	23^{g}
Senekal	-	-	45^{e}
Lichtenburg	-	61.9^{b}	-
Grand mean	62.3	43.9	47.6
L.S.D.	6.61	7.64	6.21
CV%	13	21.2	16.1

* For each variable and growing season, means followed by the same letter are not significantly different by Fischer's protected LSD test ($p \leq 0.05$).

the sunflower fields in Potchefstroom did not differ significantly (6.21) from Bothaville-Wesselsbron in Alternaria disease severity ($p \leq 0.05$). The 2014/15 season's survey showed Bothaville-Wesselsbron had the lowest disease severity mean of 17.6 % (± 7.9) in the national cultivar trials, whilst the highest mean of 76.8 % (± 6.34) was noted in Potchefstroom (locality 5) (Table 2).

The Alternaria leaf blight disease severity ratings means at the various farmers' production localities in Table 3 were not significantly different (3.8) for the three growing seasons at $p \leq 0.05$. However, the survey revealed that Alternaria leaf blight disease severity at the respective localities was significantly different. Alternaria leaf blight disease on the sunflower production fields had a mean of 47.5 % (± 14) in the 2012/13 season, with the highest at Delmas (58.7 ± 3.05) and Settlers (58.7 ± 2.08) and lowest at Viljoenskroen (18.4 ± 7.4) (Table 3). In the 2013/14 growing season, the farmer's production fields surveyed disease mean was 44.4 % (± 14) with Settlers having the highest Alternaria leaf blight mean of 79 (± 9.17) and Steinfast having the lowest mean of 24.3 (± 4.04). In the 2014/15 growing season, the Alternaria leaf blight disease mean of all the surveyed farmer fields was 45.1 % (± 9), with the highest disease severity noted in Welgelegen 60 (± 0) and the lowest in Rustenburg 3 (37.7 ± 2.52) and Vlaklaagte (37.7 ± 2.31).

The correlations between Alternaria leaf blight disease severity with environmental conditions (Annex Table S2) over the three growing seasons was determined for temperature ($r = 0.64$), relative humidity ($r = 0.15$) and rainfall ($r = 0.59$) (Table 4). Pearson's correlation coefficient between temperature and disease during the 2014/15 production season was $r = 0.84$. Temperatures between 29 and 31 °C best suited the disease proliferation of Alternaria leaf blight in the fields. However, disease severity decreased with temperatures below 27 °C.

Table 3: *The means of the Alternaria leaf blight disease severities surveyed at farmer sunflower production areas in South Africa during the 2012/13, 2013/14 and 2014/15 growing seasons.*

	Alternaria leaf blight severity (%)		
Locality	*season 2012/13*	*2013/14*	*2014/15*
Vlaklaagte	-	-	37.7^{d}
Welgelegen	-	-	60.0^{a}
Rustenburg 1	-	-	41.3^{cd}
Rustenburg 2	-	-	45.0^{bcd}
Rustenburg 3		52.7^{bc}	37.7^{d}
Lichtenburg 1	54.7^{ab}	46.7^{bcd}	58.7^{a}
Lichtenburg 2 - Coligny	-	-	40.0^{cd}
Litchtenburg 3 - Vlakfontein	-	-	46.7^{bcd}
Lichtenburg 4 - Vlakplan	-	-	55.7^{ab}
Ventersdorp	40.0^{abc}	-	49.7^{abc}
Vredefort	-	34.3^{def}	-
Vredefort	-	36.0^{def}	-
Brits	-	58.3^{b}	-
Kroonstad - Wesselsbron	-	46.7^{bcd}	-
Wesselsbron	-	24.7^{ef}	-
Settlers	58.7^{a}	79.0^{a}	-
Steinfast	-	24.3^{f}	-
Carletonville	-	41.0^{cde}	-
Delmas	58.7^{a}	-	-
Arlington	32.7^{bc}	-	-
Viljoenskroon - Rietpan	18.4^{c}	-	-
Boschpoort - Sannieshof	53.6^{ab}	-	-
Sannieshof	42.2^{ab}	-	-
Dwaalboom	42.0^{ab}	-	-
Grand mean	47.5^{A}	44.4^{A}	45.1^{A}
L.S.D.	13.52	16.63	11.15
CV%	1.2	8.3	0.3

* For each variable and growing season, means followed by the same lower case letter are not significantly different by Fischer's protected LSD test ($p \leq 0.05$). Grand means followed by the same upper case letter indicate no significant difference between the three growing seasons, where the Fisher's protected least significant difference test was 3.8 ($p \leq 0.05$) between the three growing seasons (CV % was 5.4).

The correlations between Alternaria leaf blight disease severity and relative humidity and rainfall were lower than $r = 0.53$, which makes these factors negligible and insignificant.

Table 4: *Pearson's correlations between Alternaria leaf blight of sunflower and environmental conditions (relative humidity, temperature and rainfall) in South Africa during surveys of the 2013-2015 seasons.*

Pearson Correlation Coefficients, N = 29				
	Alternaria leaf blight disease severity			
	season 2012/13	*2013/14*	*2014/15*	*mean 2013-15*
Temperature	0.619	0.712	0.840	0.644
	0.075	0.021	0.002	0.000
RH	0.054	0.342	0.521	0.150
	0.889	0.333	0.122	0.435
Rainfall	0.082	0.228	0.412	0.119
	0.832	0.527	0.236	0.539

4 Discussion

The cultivated sunflower ranks with soybean (*Glycine max* (L.) Merr.], rapeseed (*Brassica rapa* L.), and peanut (*Arachis hypogaea* L.) as one of the four most important annual crops in the world grown for edible oil (Carter, 1978; DAFF, 2017). Therefore, effective sunflower management and disease prevention practices have to be considered to ensure suitable sunflower quality and yield. Alternaria leaf blight has been reported to reduce sunflower seed and oil yield (Lagopodi & Thanasoulopoulos, 1998; Calvet *et al.*, 2005). The results of this survey generated data covering a three year period of prevalence and geographical distribution of Alternaria leaf blight in the major sunflower production regions of South Africa.

Alternaria alternata is ubiquitous in nature, occurring in different ecosystem and geographic regions (Thomma, 2003; Woudenberg *et al.*, 2015). The findings of this study supported this notion as Alternaria leaf blight was present in all surveyed sunflower farms where disease severity ranged from 44.3-47.5 % in the three growing seasons. None of the sunflower cultivars evaluated were found to be resistant to Alternaria leaf blight. Similar results were noted in the studies of van der Waals *et al.* (2001), who reported that *A. alternata* and *A. solani* causing early blight were able to infect all cultivars and were widespread in all potato and tomato growing areas. These findings were furthermore supported by other researchers reporting that *A. alternata* was able to infect all the cultivars of tangerine (*Citrus reticulata* Blanco) (Peever *et al.*, 2004) and pistachio (*Pistacia vera* L.) (Aradhya *et al.*, 2001).

This wide distribution of Alternaria leaf blight may be due to the facultative saprophytic nature that enables it to survive on dead decaying debris (Agrios, 2005); dispersal of spores by wind and other various biotic and abiotic factors due to its airborne nature (Aradhya *et al.*, 2001); contaminated seeds which serve as initial inoculum in the field during the growing season (Walcott, 2003); or toxin production which can be non-host specific and weak host selection pressures within and between populations (Aradhya *et al.*, 2001).

The site Bothaville-Wesselsbron consistently had the least Alternaria leaf blight disease severity during the 2013/14 and 2014/15 growing seasons, whereas Potchefstroom (locality 5) had the highest disease severity in all three growing seasons. This may have resulted from the farming practices (such as debris build-up, inadequate crop rotation, plant density and tillage practices) in the respective regions (Rotem, 1994; Naik *et al.*, 2007). Moreover, differences in farming practices between the cultivar trials and the farmers' fields may have resulted in the notably lower Alternaria leaf blight disease severity on the farmers' fields during the three growing seasons.

Environmental conditions play an important role in disease severity. Temperature is most important physical environmental factor for regulating the growth and reproduction of fungi (Hubballi *et al.*, 2010). In this study, Pearson's correlation coefficient between Alternaria leaf blight disease severity and environmental factors (Agrometeorology, 2015) was greatest for temperature ($r = 0.64$) as compared to rainfall ($r = 0.11$) and relative humidity ($r = 0.15$) for the three growing seasons. Studies done on Canada thistle (*Cirsium arvense* L.) demonstrated that free water was optimal, but not essential for the infection of Canada thistle by *Alternaria cirsinoxia* (Green & Bailey, 2000). Usually 10-12 hours of wetting is needed for substantial infection by *A. alternata* on citrus leaves (Timmer *et al.*, 2003), although other species require longer moisture periods of 48-72 hours (Reis *et al.*, 2006). Free moisture facilitates epidemics under all temperatures, but the higher the temperature, the quicker the spread of the epidemics by *Alternaria* species (Rotem, 1994; Reis *et al.*, 2006). This implies that after initial infection, relative humidity may cease to play a significant role in disease severity, provided that the temperature is optimal.

5 Conclusion

The distribution and prevalence of Alternaria leaf blight of sunflower was studied through surveys conducted during the 2013-2015 growing seasons. This survey showed that Alternaria leaf blight was widespread in all the major sunflower production areas of South Africa. This knowledge will play a crucial component in the sunflower crop management system because Alternaria leaf blight can be managed most effectively if control measures are introduced at an early stage of disease development (van der Waals *et al.*, 2001; Iacomi-Vasilescu *et al.*, 2004). The results of study have profound implications for future research priorities. Since there are no Alternaria leaf blight resistant sunflower cultivars; (i) field trials have to be conducted to determine the efficacy of various fungicides and cultural practices in managing Alternaria leaf blight on sunflower and (ii) proper seed tests need to be done to determine the prevalence of *Alternaria* on the seeds before planting as this may avoid the spread of inoculum on the seeds. Future studies should also aim at quantifying the damage or yield loss caused by Alternaria leaf blight of sunflower.

Ethics statement

This statement confirms that the work submitted to this journal has not been submitted elsewhere for publication, has not been published or accepted for publication, nor is being considered for publication elsewhere (either in whole or substantial part), the work is original and all authors have read the submitted version of the manuscript and approve its submission.

Supplement

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