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Control of Alternaria leaf blight caused by *Alternaria alternata* on sunflower using fungicides and *Bacillus amyloliquefaciens*

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ABSTRACT

Recent sunflower disease surveys in the major production fields of South Africa reported *Alternaria alternata* (Fries) Kiessler as the main causal agent of Alternaria leaf blight (ALB). The economic impact of ALB caused by *A. alternata* on sunflower in South Africa is unknown and there are currently no fungicide treatments registered for its control. The aim of this study was to determine the efficacy of five fungicides and one biological control agent (Integral® Pro, *Bacillus amyloliquefaciens*) as seed treatments, and three spray fungicides to control ALB on sunflower over three sunflower growing seasons (2013/14, 2014/15 and 2015/16). Alternaria leaf blight developed as early as three weeks after emergence in field trials, suggesting seed transmission of the pathogen. Alternaria leaf blight severity negatively correlated with 1000-seed weight (r = −0.33). Seed-borne *A. alternata* was significantly reduced in the field by treating seeds with Celest® XL and Dynasty® CST. Integral® Pro showed potential for future use as part of an integrated control measure for ALB of sunflower. Abacus® and Amistar® TOP were the most effective spray fungicides in reducing ALB disease severity. As ALB of sunflower has numerous possible sources of inoculum such as wind-blown inoculum, infected seed and plant debris, control should include various approaches. In this study the use of strobilurin-based fungicide seed treatments and spray fungicides both reduced ALB disease severity and also delayed sunflower leaf senescence.

1. Introduction

Sunflower (*Helianthus annuus* L.) is a member of the Asteraceae; a large family of flowering plants occurring worldwide (Weiss, 1983). In South Africa, an average of 600000 tons of sunflower grain is produced annually since 2001, mainly in the North West, Free State, Mpumalanga and Limpopo provinces (ARC-ZAR, 2018). Alternaria leaf blight (ALB) of sunflower has been reported to cause up to 80% reduction in yield by causing head rot, seedling and leaf blight (Allen et al., 1983; Lapagodi, and Thanassoulopoulos, 1998; Calvet et al., 2005). *Alternaria alternata* (Fries) Kiessler has been recently found to be the predominant species in the major production fields of South Africa during recent sunflower disease surveys causing ALB (Kgatle et al., 2019).

Alternaria leaf blight of sunflower is characterised by irregular dark, necrotic lesions with a greyish brown centre surrounded by a chlorotic halo on the leaves, stem, petioles and capitulum (Kim and Mathur, 2006). In severe infections, lesions become larger by coalescing, leading to defoliation and death of plants (Cho and Yu, 2000), resulting in a decline in the photosynthetic area that adversely affects the growth and yield of the plant (Calvet et al., 2005). In addition to leaf blight, seed infection by *Alternaria* species can result in reduction in seed production and seed germination (Prasad et al., 2009). Moreover, seedlings germinating from *Alternaria* infected seed are often blighted and may serve as the initial source of inoculum in the field (van den Berg, 2001).

The use of resistant cultivars is normally the most economical management option available against most plant diseases, unfortunately, such resistance against ALB is not usually expressed in commercially available sunflower hybrids (Mesta et al., 2011) and many other crops (Iacomi-Vasilescu et al., 2004). Other methods for managing ALB disease on other crops are based on cultural practices such as using disease-free seeds and crop rotation together with commercial fungicides (Iacomi-Vasilescu et al., 2004; Avenot et al., 2008). Fungicides, used as both seed and spray treatments, include Iprodione, Mancozeb, Carbendazim (Karuna et al., 2012), Ridomil MZ (Khan et al., 2007),

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Chlorothalonil (Kumar et al., 2013), Captan (Swart et al., 1998) and strobilurin based fungicides (pyraclostrobin and Amistar®) (Horsfield et al., 2010).

There are currently no registered fungicides for the control of ALB on sunflower. This study aimed to determine the efficacy of five seed treatment fungicides, three spray fungicides and a biological control agent against ALB in three seed treatment and three fungicide spray program trials. The rational was to find a broad spectrum of fungicides that can control ALB of sunflower, with the aim of integrating these fungicides with other cultural ALB disease management practices.

2. Materials and methods

2.1. Production practices

Field trials were conducted during the 2013/14, 2014/15 and 2015/16 growing seasons at the experimental site of the Agricultural Research Council-Grain Crops Institute in Potchefstroom which is situated at 26.745°S, 27.083°E, 1322 m above sea level in the North West province, South Africa. In each respective growing season, one seed treatment and one spray program trial was conducted.

Conventional cultural practices of commercial fields were used, which included a standard application of 150 kg/ha blending fertilizer (3N:2P:1K (25) + 0.5% Zn) incorporated into the seedbed before planting. Euro-Lightning® (BASF, Midrand) was sprayed as a post-emergence herbicide against broadleaf and grass weeds. Trials were planted under dry land conditions and sprinkle irrigation was done whenever necessary. Seeds of the test cultivars PAN 7057 and PAN 7351 were sown manually at 1.5–2 kg/ha for both seed treatment and spray program trials. Two to three weeks after emergence, seedlings were thinned to 18 plants per row. Each row was 6 m long, with an inter-row spacing of 1 m and a 0.35 m intra-row spacing was used in all sites during the three growing seasons. In all the field trials, seed lots of cultivars PAN 7057 (74% infection) and PAN 7351 (57% infection) which were naturally infected with *A. alternata* were used for planting..

2.2. Seed treatments

Seed treatment field trials consisted of five fungicide treatments, Dividend® 030 FS (Syngenta, Midrand, South Africa), Galmano® 167 FS (Bayer, Centurion, South Africa), Apron® XL (Syngenta, Midrand, South Africa), Celest® XL (Syngenta, Midrand, South Africa) and Dynasty® CST (Syngenta, Midrand, South Africa), and a registered biological control agent Integral® Pro (*Bacillus amyloliquefaciens* MBI600) (BASF, Midrand, South Africa) (Table 1) applied at a recommended and double dosage rate, and an untreated control. The seed treatment field trials were conducted using a completely randomised block design with six replications. Each treatment consisted of three rows. Three rows of sunflower were used as a border in between each replication.

2.3. Fungicide sprays

Fungicide spray field trials consisted of three treatments, Duett® (BASF, Midrand), Abacus® (BASF, Midrand) and Amistar® TOP (Syngenta, Midrand) (Table 1). Celest® XL (Syngenta, Midrand) showed efficacy in reducing Alternaria seed infection during preliminary laboratory tests and was therefore used for treating all the seed that were used in fungicide spray field trials. The fungicide spray trials were conducted using a completely randomised block design with four replications. Each treatment consisted of five rows. The spray schedule included three spray applications of each treatment from the sixth week at a four week intervals. Spray fungicides were applied at recommended rates using a four nozzle knapsack sprayer (Efekto, Midrand, South Africa). Five rows of sunflower were used as a border in between each replication.

2.4. Harvesting, yield seed mass and 1000-seed weight

Flower heads from the middle row (18 plants) of each seed treatment trial and the middle two rows (36 plants) of each spray program plot were harvested and evaluated in the subsequent tests for ALB. Flower heads were harvested and dried in the sun for 3–5 days. Seeds were threshed from heads manually and dried at room temperature (25 °C) for a month. Subsequent to drying, the yield seed mass and 1000-seed weight of each treatment in each replicate was determined.

2.5. Standard germination tests

After harvesting, the standard germination test was conducted for all the treatments from all seed treatment and spray program field trials using the between-paper method modified from the International Seed Testing Association rules (ISTA, 2018). Two hundred (four replicates of 50) sunflower seeds from each treatment were randomly selected and placed on three layers of germination paper towels (Agricol (Pty) Ltd, Midrand, South Africa) and then covered with a fourth layer. The paper towels were moistened with distilled water. The paper towels were loosely rolled, placed in polyethylene bags and sealed with an elastic band. The bags were incubated in an upright position in an incubator at

Table 1
List of fungicides used against Alternaria leaf blight of sunflower during seed treatment and spray program field trials.

Trade name	Active ingredient (s)	Fungicide class	Formulation	Spectrum	Recommended dosage rate
Seed treatment					
Dividend® 030 FS	Difenonazole (30 a.i. g/l)	Phenylamides	Seed coat	broad – spectrum, systemic	200 ml/100 kg
Galmano® 167 FS	Fluquinconazole (167 a.i. g/l)	Triazole	Seed coat	broad - spectrum	450 ml/100 kg
Apron® XL	Metalaxyl-M (350 a.i. g/l)	Phenylamides	Seed coat	Oomycetes	1000 ml/100 kg
Celest® XL	Fludioxonil (25 a.i. g/l) and Mefenoxam (10 a.i. g/l)	Phenylpyrrols and Phenylamides	Seed coat	broad – spectrum, systemic	150 ml/100 kg
Dynasty® CST	Azoxystrobin (10 a.i. g/l), Fludionoxil (10 a.i. g/l) and Metalaxyl-M (10 a.i. g/l)	QoI (Quinone outside inhibitors)	Seed coat	broad – spectrum, systemic	150 ml/100 kg
Integral® Pro	Biological control agent (BCA)	*Bacillus amyloliquefaciens* MBI600	Seed coat	broad – spectrum, systemic	150 ml/100 kg
Spray program					
Duett®	Epoxyconazole (125 a.i. g/l) and Carbendazim (125 a.i. g/l)	Triazole and benzimidazole	Foliar spray	broad – spectrum, systemic	800 ml/ha
Abacus®	Epoxyconazole (62.5 a.i. g/l) and pyraclostrobin (62.5 a.i. g/l)	QoI (Quinone outside inhibitors)	Foliar spray	broad – spectrum, systemic	1000 ml/ha
Amistar® TOP	Azoxystrobin (200 a.i. g/l) and difenoconazole (125 a.i. g/l)	QoI (Quinone outside inhibitors)	Foliar spray	broad – spectrum, systemic	500 ml/ha

25 °C. The first and second count of germination was made after 4 and 10 days. Results were presented as the percentage normal seedlings at the end of the test period.

2.6. Seed health test

Two hundred seeds of each treatment were surface-disinfected with 1% sodium hypochlorite for 5 min, rinsed with sterile distilled water and placed on a sterile paper towel in the laminar flow until dry. Five seeds were evenly spaced on potato dextrose agar (PDA) (Biolab, Merck, Modderfontein, South Africa) in each Petri dish. The plated seeds were incubated for 5–7 days at 25 °C under 12 h alternating cycles of ultraviolet light and darkness (Mathur and Kongsdal, 2003). *Alternaria*-infected seeds were counted to determine the total number of infected seeds.

2.7. Rating and data analysis

Sunflower leaves were visually rated for ALB disease on each plant every two weeks using a scale of 0–4 suggested by Allen et al. (1983) where 0 = no disease; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100% of the plant showing ALB. Seven disease severity ratings were done during the growing seasons from the fourth week after planting. Only the middle row of each seed treatment trial and the middle two rows of each spray program trial were assessed for ALB disease severity.

After harvesting and threshing seeds, the ALB disease parameters (AUDPC, seedling blight and seed infection) and sunflower germination, yield seed weight and 1000-seed weight of each treatment were recorded and a factorial analysis of variance was done using GenStat 64-bit Release 18.2 (VSN International Ltd) to compare treatments. Correlation analyses between ALB disease parameters and sunflower germination, yield seed weight and 1000-seed weight were determined by Pearson's correlations using the SAS v9.4 (SAS Institute, Inc., 2013) statistical package. The efficacy of each treatment in reducing ALB was furthermore calculated as:

$$R = [1 - T/C] \times 100$$

Where, R = Disease reduction percentage.

T = Alternaria disease severity percentage of treatment
C = Alternaria disease severity percentage of untreated control (%)

Table 2
The efficacy of various seed-treatment fungicides on Alternaria leaf blight disease parameters (seed infection, seedling blight and disease severity) and sunflower germination, yield seed mass and 1000-seed weight during the 2013/14–2015/16 sunflower field trials.

Year	Seed treatment	Seedling blight (%)	Seed infection (%)	Germination (%)	Seed yield (g)	1000-seed weight (g)	AUDPC
2013/2014	Dividend®	9.8 ab	55.9 ab	88.2 de	253.1	49.5 c	222.5 b
	Dividend® (x2)	9.7 ab	52.7 abc	91.2 abcd	229.4	51.4 bc	218.1 b
	Galmano®	9.2 ab	52.8 abc	87.8 e	238.8	52.3 bc	217.3 b
	Galmano® (x2)	9.7 ab	51.7 bc	90.8 abcde	227.5	51.3 bc	215.8 b
	Apron® XL	10.1 ab	55.0 ab	91.7 abc	181.8	48.9 c	222.9 b
	Apron® XL (x2)	10.0 ab	57.8 a	89.8 bcde	252.0	51.4 bc	214.1 bc
	Dynasty® CST	6.2 cb	48.3 c	91.5 abc	268.0	54.4 ab	194.5 d
	Dynasty® CST (x2)	4.8 d	49.1 c	92.7 ab	297.9	54.4 ab	202.8 cd
	Celest® XL	4.1 d	49.1 bc	92.2 abc	326.4	50.6 bc	192.7 d
	Celest® XL (x2)	5.1 d	48.6 c	93.9 a	280.9	56.3 a	198.6 d
	Integral® Pro	8.3 bc	55.0 ab	91.3 abcd	271.9	51.6 bc	214.9 bc
	Untreated control	10.8 a	57.8 a	89.2 cde	242.6	49.3 c	236.6 a
	Grand mean	**8.12**	**52.81**	**90.83**	**255.9**	**51.76**	**212.6**
	L.S.D.	**2.433**	**5.914**	**3.298**	**ns**	**3.884**	**12.63**
	Cv%	**11.5**	**4.9**	**2.6**	**14.7**	**4.3**	**2.3**
2014/2015	Dividend®	7.6 abcd	57.9 abc	92.6	649.0 cd	46.6 def	188.6 c
	Dividend® (x2)	9.6 a	58.4 abc	90.8	620.9 d	49.7 bcde	194 bc
	Galmano®	8.1 abc	57.7 abc	92.2	702.6 bcd	48.4 bcdef	195.9 bc
	Galmano® (x2)	7.3 bcd	61.3 ab	90.7	767.5 abcd	45.7 ef	187.6 c
	Apron® XL	9.4 ab	60.9 ab	91.7	764.4 abcd	47.6 cdef	192.2 bc
	Apron® XL (x2)	8.8 ab	55.6 abcd	92.5	809.4 abc	50.1 abcd	199.8 ab
	Dynasty® CST	61 cd	44.9 e	93.9	872.8 ab	51.0 abc	172.6 d
	Dynasty® CST (x2)	7.8 abcd	53.8 bcde	92.8	783.7 abcd	54.1 a	170.1 d
	Celest® XL	5.5 d	47.1 de	91.1	866.1 ab	52.4 ab	170.2 d
	Celest® XL (x2)	6.1 cd	51.2 cde	92.8	898.7 a	51.8 abc	171.5 d
	Integral® Pro	8.1 abc	55.5 abcd	91.9	628.6 d	48.3 bcdef	188.6 c
	Untreated control	7.7 abcd	63.2 a	92.6	766.1 abcd	44.4 f	209.7 a
	Grand mean	**7.67**	**55.61**	**92.13**	**757**	**49.19**	**186.7**
	L.S.D.	**2.32**	**9.31**	**-**	**176.6**	**4.25**	**10.98**
	Cv%	**5.1**	**10.4**	**0.7**	**6**	**7.4**	**1.8**
2015/2016	Dividend®	9.667 a	54.92 ab	91.92 abc	689.9 bc	48.09 f	106.3 bc
	Dividend® (x2)	9.167 ab	52.25 bc	92.42 ab	566.2 c	50.09 def	105.6 bc
	Galmano®	7.333 abcd	50.42 bc	92.08 abc	582 c	52.67 abcde	108.6 b
	Galmano® (x2)	8.917 abc	50.17 bc	90.33 bc	522.2 c	50.12 def	106.2 bc
	Apron® XL	8.667 abc	59.58 a	92.83 ab	496.9 c	50.32 def	104.8 bc
	Apron® XL (x2)	8.417 abc	50.5 bc	89.5 c	680.6 bc	50.98 cdef	106.6 bc
	Dynasty® CST	5.417 d	53.75 ab	93.58 a	883.5 ab	53.82 abc	94.7 d
	Dynasty® CST (x2)	6.333 cd	47.17 c	93.75 a	865.7 ab	54.67 ab	97.1 d
	Celest® XL	6.667 bcd	47.42 c	93.25 a	1029.4 a	53.56 abcd	95.7 d
	Celest® XL (x2)	5.5 d	50.67 bc	93.5 a	850.7 ab	54.74 a	100.1 cd
	Integral® Pro	8.5 abc	54.58 ab	93 a	566 c	51.24 bcdef	106.1 bc
	Untreated control	9.083 ab	55.67 ab	91.83 abc	620.4 c	49.57 ef	117.3 a
	Grand mean	**8.12**	**52.26**	**92.33**	**696**	**51.76**	**104.10**
	L.S.D.	**2.642**	**6.244**	**2.664**	**213.6**	**3.478**	**6.997**
	Cv%	**9**	**2**	**0.6**	**7.2**	**3.3**	**3.1**

Significant at $P < 0.05$ Fisher's protected least significant value, ns = not significant. The (x2) represents double dosage of the fungicide.

3. Results

In all three seasons, sporadic initial ALB symptoms were observed on the cotyledons from 3 to 4 weeks after emergence in the fields in the seed treatment trials, mostly notable on the untreated control.

3.1. Seed treatments

Results indicated that the efficacy of the seed treatment using Celest® XL and Dynasty® CST fungicides differed significantly when compared to the untreated control ($P \leq 0.05$) (Table 2). The average seed germination was 90.8% during the 2013/14 season with no significant difference between the cultivars PAN 7057 (91%) and PAN 7351 (90.7%). Some of the germinated seedlings were infected with *A. alternata*. Although the untreated control (10.8%) had the highest seedling blight infection, there were no significant differences when compared to seed treatments with Dividend® XL (9.8%), Galmano® (9.2%) and Apron® XL (10.1%). Furthermore, the untreated control had the highest seed infection (57.8%). Contrariwise, Celest® XL (56.3 g) and Dynasty® CST (54.4 g) seed treatments had the highest 1000-seed weight and the least seed infection. There was no significant difference in yield seed weight among the seed treatments.

There was no significant difference in germination among the various seed treatments and the control with the average germination of 92.1% during the 2014/15 growing season (Table 2). Seeds treated with Celest® XL (5.5%) had the least seedling blight infection whereas those of Dividend® XL (9.6%) had the highest seedling blight post-germination. The untreated control had the highest seed infection (63.2%) although it was not significantly different to seed treated with Apron® XL, Galmano®, Dividend® XL and Integral® Pro. Furthermore, the untreated control had the highest disease severity of 209.7. Seeds treated with Dynasty® CST and Celest® XL had the lowest *Alternaria* disease severity, seedling blight infection and the highest 1000-seed weight.

During the 2015/16 growing season, the average seed germination was 92.3% and there was no significant difference among the seed treatments when compared to the untreated control at $P < 0.05$ (Table 2). The average *Alternaria* disease severity and seedling blight was 104.1 and 8.1%, respectively. Seeds treated with Dynasty® CST (94.7) and Celest® XL (95.7) had the lowest disease severity. Seeds treated with Apron® XL (59.6%) had the highest seed infection, although not significantly different from those of the untreated control (55.7%), Dividend® XL (54.9%), Dynasty® CST (53.8%) and Integral® Pro (54.6%).

Five seed treatment fungicides and Integral® Pro, a biological control agent, showed different levels of disease severity (AUDPC) where disease severity decreased in the three years of field trials, with the ANOVA indicating a significant difference during the three growing seasons. The mean AUPDC for 2013/14, 2014/15 and 2015/16 seed treatment field trials was 212.6, 186.7 and 104.1, respectively, where the least significant difference was 3.01 at $P \leq 0.05$. Celest® XL and Dynasty® CST seed treatments showed a better reduction in ALB disease parameters. This trend was observed in all field trials during the three growing seasons. Although the untreated control had the highest ALB disease severity, there were no significant differences when compared to seed treatments such as Dividend® XL, Galmano® and Apron® XL. In all the treatments, there were no significant differences between the recommended and the double dosages.

The mean seed infection for 2013/14 growing season was 55.6%, which was significantly different from the 2014/15 and 2015/16 growing seasons, which had seed infections of 52.8% and 52.3%, respectively, with a LSD of 2.33 ($P \leq 0.05$) (Table 2). Results indicate that there was no significant difference in 1000-seed weight across the three growing seasons, namely 51.8 g, 51.7 g and 49.2 g, respectively. There was no significant difference in seedling blight infection and germination over the three growing seasons.

The average ALB disease reduction of the most effective seed treatment fungicides during the three field trials, compared to the untreated control, was Celest® XL where seedling blight, seed infection and AUDPC was reduced by 28%, 25% and 18%, respectively. Dynasty® CST reduced seedling blight, seed infection and AUDPC by 20%, 29% and 18%, respectively. The Integral® Pro biological control agent was the third most effective seed treatment that showed significant differences in certain instances.

3.2. Fungicide sprays

All spray fungicide treatments were significantly different from the control regarding seedling blight at $P \leq 0.05$ during the 2013/14 spray program field trials (Table 3). However, there was no significant difference in seed infection, 1000-seed weight and yield seed weight. Seeds harvested from plants sprayed with Abacus® (93.9%) and Duett® (94.1%) had the highest germination, while seeds from plants sprayed with Amistar® TOP did not differ from the control.

During the 2014/15 growing season, the Duett® spray treatment was not significantly different to the Celest® XL seed treated control regarding germination, yield seed weight and 1000-seed weight and disease parameters (except for seed infection). The control was furthermore not significantly different from Abacus® and Amistar® TOP spray fungicides regarding seed germination, yield mass and 1000-seed weight. The Abacus® spray treatment was however significantly different from the control regarding disease severity and seedling blight infection.

During the 2015/16 spray program trial, the spray treatments were not significantly different from the Celest® XL seed treated control regarding germination, seedling blight, seed infection and yield seed weight. Seed from the Abacus® spray treatment (61.3 g) was significantly different from the control (54.9 g) regarding 1000-seed weight. The control had the highest disease severity (102.0).

Three spray fungicide treatments showed different levels of disease severity (AUDPC). Disease severity decreased in the three years of field trials, with the ANOVA indicating a significant difference during the three growing seasons. The mean AUPDC for 2013/14, 2014/15 and 2015/16 fungicide spray field trials was 188.7, 167.8 and 96.6, respectively, where the least significant difference was 10.35 at $P \leq 0.05$. Sunflowers sprayed with Abacus® and Amistar® TOP had a significantly lower disease severity (AUDPC) than the Celest® seed treated control over the three fungicide spray field trails and decreased ALB.

3.3. Pearson's correlations

Pearson's coefficient determined the correlation of ALB disease parameters (seed infection, disease severity and seedling blight) with sunflower germination, yield seed weight and 1000-seed weight (Table 4). There was a high negative correlation ($r = -0.83$) between seed infection and 1000-seed weight. This demonstrates that there is a direct relationship between these two variables, which means seed-lots with a high *Alternaria* infection will generally have a lower seed weight. Seed germination was negatively correlated to disease severity ($r = -0.53$) and seedling blight ($r = -0.5$).

4. Discussion

Alternaria leaf blight disease severity has been negatively correlated with yield and has been known to significantly reduce head diameter, number of seeds produced per head, 1000-seed weight and percentage oil content (Lapagodi, and Thanassoulopoulos, 1998). To date little research has been done in South Africa to evaluate fungicide treatments for the control of ALB of sunflower. Thus, seed treatment field trials (consisting of a biological control agent and five fungicides) and spray program field trials (consisting of three spray fungicides) were carried

Table 3
The efficacy of various spray programme fungicides on Alternaria leaf blight disease parameters (seed infection, seedling blight and disease severity) and sunflower germination, yield seed mass and 1000-seed weight during the 2013/14–2015/16 sunflower field trials. Significant at P < 0.05 Fisher's protected least significant value, ns = not significant.

Year	Seed treatment	Seedling blight (%)	Seed infection (%)	Germination (%)	Seed yield (g)	1000-seed weight (g)	AUDPC
2013/14	Duett®	4.25 b	47.1	94.12 a	451	54.77	186.1 b
	Abacus®	4.5 b	42.6	93.88 a	503	58.59	180.7 b
	Amistar® Top	4.375 b	53.1	88.38 b	383	56.79	176.7 b
	Control (Celest® XL)	7.875 a	51.9	89.75 b	627	54.22	199.72 a
	Grand mean	**5.25**	**48.7**	**91.53**	**491**	**56.09**	**188.7**
	L.S.D.	**2.322**	**ns**	**2.896**	**ns**	**ns**	**16.21**
	Cv%	**26.2**	**6.5**	**1.7**	**16.7**	**5.8**	**1.4**
2014/15	Duett®	5.88 a	49 b	91.25	921	54.93	170 a
	Abacus®	3.75 b	44.88 b	94.5	1026	53.65	158.5 b
	Amistar® Top	6.88 a	47.38 b	93.38	1080	51.01	161.1 b
	Control (Celest® XL)	5.5 a	62.38 a	92.38	914	51.36	172.7 a
	Grand mean	**5.5**	**50.9**	**92.88**	**985**	**52.74**	**167.8**
	L.S.D.	**2.311**	**8.26**	**ns**	**ns**	**ns**	**9.52**
	Cv%	**15.9**	**8.6**	**1.7**	**8.8**	**5.5**	**3.4**
2015/16	Duett®	9.88	52.6	92.25	748	57.65 ab	96.93 b
	Abacus®	5.5	51.9	94.75	1101	61.31 a	91.78 c
	Amistar® Top	7.5	52.4	92.75	917	56.67 b	95.48 bc
	Control (Celest® XL)	5.88	44.1	93.75	862	54.91 b	102.01 a
	Grand mean	**7.19**	**50.2**	**93.38**	**907**	**56.09**	**96.55**
	L.S.D.	**ns**	**ns**	**ns**	**ns**	**4.478**	**4.847**
	Cv%	**15.7**	**3.7**	**1.5**	**23.3**	**4.7**	**2.5**

Table 4
Pearson's correlation coefficients between Alternaria leaf blight disease parameters and sunflower germination, yield seed weight and 1000-seed weight.

Pearson correlation coefficients, N = 21 Prob > |r| under H0: Rho = 0	Germination	Yield seed weight	1000-seed weight
Seedling blight	−0.5	−0.33	0.08
	0.02	0.1415	0.72
Seed infection	−0.35	−0.02	−0.83
	0.121	0.9235	<.0001
AUDPC	−0.53	−0.57	−0.33
	0.0137	0.0067	0.15

out to determine the relative efficacy against ALB during three sunflower growing seasons.

Alternaria leaf blight was noticed as early as the third week after emergence and occurred throughout the season. The early sporadic initial infection in the field may have been caused by the inoculum in the seeds. PAN 7057 had higher *Alternaria* seed infection (74%) than PAN 7351 (57%) prior to planting; consequently PAN 7057 had a higher *Alternaria* disease severity (AUDPC) (54.9%) than PAN 7351 (52.2%) in the field trials and post-harvest laboratory tests. *Alternaria* seed infection is of great significance from a plant quarantine perspective as this pathogen can be transmitted from one location to another through seeds (Girish et al., 2007).

Seed treatments with the lowest efficacy against ALB of sunflower were Apron® XL (mefenoxam), Dividend® XL (difenoconazole and Apron® XL) and Galmano® (fluquinconazole and prochloraz). Apron® XL and Dividend® XL seed treatments have been shown to be highly effective against many seed rot and damping-off diseases caused by Oomycete pathogens, including *Pythium* and *Phytophthora* species (Syngenta, 2010). Galmano® is a triazole seed treatment fungicide that inhibits sterol biosynthesis with a relatively broad spectrum of activity (Wu et al., 2001; Freeman et al., 2005) and has been previously reported to reduce Alternaria leaf blight of *Pseudopanax* at a concentration of 10 mg/l (Everett and Neilson, 1996). The lack of efficacy in this study may be attributed to the concentration of the active ingredient.

Celest® XL (fludioxonil and difenoconzale) and Dynasty® CST (azoxystrobin, fludioxonil and difenoconzale) seed treatments significantly reduced ALB disease severity compared to the untreated control. Celest® XL and Dynasty® CST have been shown to suppress the growth of *Alternaria* by inhibiting spore germination (Iacomi-Vasilescu et al., 2004; Akgule et al., 2011). Furthermore, Dynasty® CST contains azoxystrobin that inhibits mitochondrial respiration in the fungal cell, thus preventing energy production (Syngenta, 2006).

Application with Celest® XL and Dynasty® CST can be detected in internal seed tissues and the coleoptiles of germinating seeds which is important in treating seed-borne *Alternaria* species (Lagopodi and Thanassoulopoulos, 1998; Munkvold and O'Mara, 2002). The disadvantage of using seed treatments for controlling ALB is that fungicides applied on seeds normally provide protection only during germination, emergence and the early establishment stage of the plant due to the presence of relatively small amounts of fungicide applied on the seeds (Birah et al., 2014). Furthurmore, inoculum can be easily introduced into the field by wind dispersal and rain splashes (Birah et al., 2014; Woudenburg et al., 2015).

Alternaria leaf blight was observed to reduce the amount of green leaf area during the field trials. Crops such as sunflower have a determinate number of leaves. Therefore, a greater Alternaria leaf blight disease severity will result in the decrease in sunflower yield (Leite et al., 2006). The loss of leaves during the field trials could therefore affect the nett photosynthesis of the remaining green leaf tissue, thus decreasing the supply of photosynthates to the various sink organs and affecting processes such as grain filling (Lagopodi and Thanassoulopoulos, 1998; Calvet et al., 2005). The correlation between Alternaria disease severity and 1000-seed weight was $r = -0.33$. Abacus® (pyraclostrobin) and Amistar® TOP (azoxystrobin and difenoconazole) contain strobilurin which has been previously shown to induce growth stimulation, hormonal changes and delayed senescence that contribute to increased yields (Grichar, 2013; Mahoney et al., 2015). In essence, this study shows that strobilurin-treated (Abacus® and Amistar® TOP) sunflower plants displayed prolonged greening that allows for greater dry matter accumulation through the seed-filling period and higher yields as more photosynthetic efficient leaves are retained longer (Mahoney et al., 2015). Strobilurin based Abacus® (pyraclostrobin) and Amistar® TOP (azoxystrobin and difenoconazole) have a mode of action that inhibits mitochondrial respiration, preventing spore germination, reducing mycelial growth and are active against many plant pathogenic fungi (Mahoney et al., 2015).

The results of this study concur with the findings of Lagopodi and Thanassoulopoulos (1998) where *A. alternata* was found to have the potential to be as damaging to sunflower as *A. helianthi*. The control of ALB of sunflower can be achieved by implementing practices such as crop rotation; removal of plant debris; and the use of disease free seeds that minimise inoculum introduction into the field together with the use of fungicides. The alternation of seed treatments and field spray programs with fungicides such as Dynasty® CST, Celest® XL, Duett®, Amistar® TOP and Abacus® can be recommended since they significantly reduced ALB. These fungicides have different modes of actions and can therefore reduce chemical resistance risk.

While chemical applications are still the main method in controlling Alternaria leaf diseases of many crops, chemicals can be harmful to the environment and frequent treatments using fungicides with the same mode of action could result in fungicide resistance among pathogen populations ultimately resulting in disease-control failures (Avenot et al., 2008). Integral® Pro (*B. amyloliquefaciens* MBI600) showed potential in reducing ALB. *Bacillus* is a plant growth promoting rhizobacterium reported to colonize the roots and is capable in promoting plant growth (O'Callagham, 2016; Qi et al., 2016). The use of Integral® Pro for seed treatments should therefore be investigated further. Although spray treatments showed a high efficacy in reducing ALB, spray fungicides may not be a viable option for sunflower producers to use for controlling *Alternaria,* due to financial implications. These fungicides can however be recommended for seed production companies to reduce levels of seed infection.

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 approved its submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

M.G. Kgatle: Investigation, Methodology, Formal analysis, Writing - original draft. **B. Flett:** Conceptualization, Methodology, Formal analysis, Writing - review & editing, Project administration, Supervision. **M. Truter:** Validation, Writing - review & editing, Visualization, Supervision. **T.A.S. Aveling:** Conceptualization, Formal analysis, Resources, Data curation, Writing - review & editing, Project administration, Funding acquisition, Supervision.

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