Effect of irrigation on charcoal rot severity, yield loss and colonization of soybean and sunflower

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Highlights

• Irrigation did not prevent infection by *M. phaseolina,* but reduced disease severity.

• Increased colonization of sunflower plants by *M. phaseolina* resulted in higher yield loss.

• Microsclerotia concentration was higher in soybean than sunflower.

Abstract

Charcoal rot incidence in soybean and sunflower in South Africa is increasing. Irrigation as a means to manage charcoal rot is limited as water resources are decreasing and oil seed production is predominantly on dry land. The effect of reduced soil moisture on charcoal rot incidence, severity and colonization of host tissues, as well as subsequent yield loss, was investigated under optimal and sub-optimal conditions for disease development on hosts grown in rotation with *Zea mays* (maize). Pot experiments were carried out in a greenhouse using a locally planted *Glycine max* (L.) Merr. (soybean) and *Helianthus annuus* L. (sunflower)

cultivar, which were inoculated with *Macrophomina phaseolina* (Tassi) Goid. isolated from sunflower grown in a major oil seed-producing region in South Africa. The disease index, calculated as the product of incidence and severity divided by the total number of observations made per treatment, ((incidence*severity)/(total number of observations made per treatment)), was 20% higher in soybean than in sunflower, even though colonization of sunflower, calculated as the frequency of *M. phaseolina* isolated from the stem section between the roots and the cotyledon, was 10% more than that of soybean. *M. phaseolina* was not isolated past the first node of either crop. Yield loss due to charcoal rot amounted to 6.13% in soybean and 12% in sunflower, relative to the uninoculated, reduced soil moisture controls. Microsclerotia were more concentrated in soybean than in sunflower, which indicates that a soybean crop could increase soil inoculum more readily than sunflower. Consideration should therefore be given to the charcoal rot susceptibility of the crop following soybeans. Reduced soil moisture and infection were synergistic to disease incidence and yield loss, but independent of colonization and disease severity. Results showed that an increase in soil moisture cannot prevent initial infection of the host, but can significantly reduce colonization of the stem at maturity.

Keywords: *Macrophomina*, irrigation, severity, colonization, soybean, sunflower

1. Introduction

Soybean (*Glycine max* (L.) Merr.) and sunflower (*Helianthus annuus* L.) oil, used in protein cakes, are major sources of animal feed in South Africa (DAFF, 2018). During the 2017/2018 soybean growing season, the country produced a record 2.29 t/ha (DAFF, 2018; Grain SA, 2018). During the same season, sunflower production was estimated at 1.38 t/ha.

The major soybean-producing regions include the Mpumalanga and Free State provinces, while sunflower is also predominantly produced in the Free State, followed by the North West province. Both oil seed crops are often grown under dry land conditions and in rotation with one another as well as with cereal crops, notably maize. Projections for increasing yields have been made by the voluntary association of grain farmers in South Africa, also known as Grain South Africa, but these yield increases are hampered by the water scarcity in the country (Sihlobo and Kapuya, 2016).

Diseases such as charcoal rot, which is associated with drought, have previously gone unnoticed, but can result in severe yield losses. The first report of charcoal rot in South Africa on soybeans was by Jooste (1969). By this time, however, the disease had already been reported on sunflowers (Jooste, 1969). Symptoms of charcoal rot usually appear at the end of the season, following flowering (Ijaz et al., 2012; Kaur et al., 2012; Luna et al., 2017). Typical symptoms include stunting, chlorosis and wilting. It also leads to reduced yields and early senescence (Kaur et al., 2012; Khan, 2007; Luna et al., 2017). When soybeans are infected, the leaves remain attached to the petioles (Luna et al., 2017; Mengistu et al., 2007). The name charcoal rot relates to the formation of microsclerotia at the base of the stems, giving them a grey appearance (Kaur et al., 2012; Purkayastha et al., 2006).

Charcoal rot is caused by *Macrophomina phaseolina* (Tassi) Goid., a fungus that exhibits host preference rather than specificity and has acquired a host range of more than 500 plant species (Mahmoudi and Ghashghaie, 2013; Su et al., 2001; Zveibil et al., 2012). Isolates from one host will often cause disease in another (Su et al., 2001; Zveibil et al., 2012; Luna et al., 2017). In most cases it is difficult to distinguish the primary from the secondary host, as they are grown in rotation. Host specialization studies are largely based on the isolation of *M. phaseolina* from various hosts and the inoculation of these isolates into their host of origin as well as other hosts (Su et al., 2001). These studies have concluded that no host specialization

was observed with isolates from soybean, sorghum or cotton and that colonization of soybean was preferred over sorghum and cotton, even when inoculated with isolates not originally obtained from soybean. Intercropping and crop rotation results in an increase in sclerotia.

The primary source of inoculum is microsclerotia that act as survival structures and are capable of germination within two days in the vicinity of roots; however, germination can persist throughout the growing season (Chowdhury et al., 2014; Kendig et al., 2000). Infection of the roots occurs very early in the season, but factors affecting root colonization rate are not completely understood (Almeida et al., 2003; Kendig et al., 2000; Luna et al., 2017). At seedling stage, *M. phaseolina* infects and remains in the plant tissue until conditions are optimal for disease development, usually when additional stress on the plant makes it more susceptible to the pathogen (Gupta et al., 2012). Temperatures of 25°C to 30°C and extended periods of dry weather (soil moisture below 60%) are optimal for disease development (Dhingra and Sinclair, 1975; Pearson et al., 1984; Purkayastha et al., 2006). Sexton et al. (2016) compared soybean isolates from the northern and southern regions of the United States of America and concluded that *M. phaseolina* isolates were regionally adapted. This suggests that charcoal rot should be studied using isolates from within a given area and under local environmental conditions.

At present, there is no reliable method for controlling charcoal rot. Due to the wide host range of *M. phaseolina*, crop rotation is not always effective. A few soybean cultivars from maturity groups IV and V, and some sunflower varieties, have shown some resistance to drought and charcoal rot; however, resistance in soybean and sunflower has not been recorded (Luna et al., 2017; Mengistu et al., 2011a; Smith and Wyllie, 1999). In South Africa, the susceptibility of varieties to charcoal rot has not been documented or investigated.

Another effective means of managing charcoal rot is irrigation. The premise of this management strategy is based on increasing the soil moisture and thus minimizing host stress during dry conditions and during critical stages of stress, such as flowering, when translocation stress is expected to increase susceptibility to colonization (Dodd, 1980; Kendig et al., 2000; Shokes et al., 1977). Increased soil moisture also reduces sclerotial germination and decreases chances of infection (Dhingra and Sinclair, 1975). Unfortunately, in South Africa, water is a scarce and costly resource to consider as a management option.

Charcoal rot is not generally considered a major disease of soybeans or sunflowers in South Africa, but is starting to gain interest in the industry due to increasing drought conditions. Most oil seed crop-growing regions in South Africa reach average temperatures needed for charcoal rot development during the growing season. It is expected that, due to the changing climate, the average temperatures will increase by 2°C in future and that annual rainfall will decrease (Van der Waals et al., 2013; Ziervogel et al., 2014), thus increasing the frequency of optimal conditions for development of charcoal rot. Reports of 40% to 70% yield loss due to charcoal rot in South African soybean and maize fields in the 2007/2008 and 2012/2013 growing seasons show the devastating effect that the disease can have (Coleman, 2013; Janse van Rensburg and Flett, 2012). These disease outbreaks have made growers more aware of how charcoal rot could potentially affect soybean and sunflower crops, particularly as these crops are frequently grown in rotation with maize, leading to a build-up of inoculum in the soil (DAFF, 2018; Luna et al., 2017). A survey conducted by the Agricultural Research Council of South Africa in 2012 identified charcoal rot in all of the oil seed-producing regions, except KwaZulu-Natal (Tewoldemedhin and Lamprecht, 2015). Information regarding yield losses due to charcoal rot in South Africa is currently limited. A major obstacle in determining yield loss directly related to *M. phaseolina* infection is the inability to separate reduced soil moisture (RSM) and disease incidence in a given season under field conditions. There are also no

fungicides or fumigants that can eradicate the inoculum in the soil, and as such, non-inoculated controls are often difficult to obtain in field trials. This study investigated the interaction between RSM and a *M. phaseolina* isolate from sunflower in terms of charcoal rot development within the host of origin (sunflower), as well as on soybean, a host frequently grown in rotation with sunflower. Disease index and stem colonization at the seedling, flowering and maturity growth stages, and subsequent yield loss were assessed in pot trials done under controlled greenhouse conditions.

2. Materials and methods

2.1 Inoculum preparation

Inoculum was prepared according to Mengistu et al. (2011a); in brief, millet seeds were soaked for 20 hours in a solution containing 40 g table sugar, 0.5 g yeast extract and 0.25 g tartaric acid per litre of sterile distilled water. The liquid was removed and seeds were distributed into 250 ml Erlenmeyer flasks (15 g millet seed per flask) that were subsequently plugged with cotton wool and autoclaved at 121°C for 30 min. An isolate of *M. phaseolina*, isolated from sunflower from the Free State during 2015, was used in the experiment. This isolate was deposited into the Plant Protection Research culture collection with the Accession Number PPRI 23755 (Agricultural Research Council, Pretoria, South Africa). Pathogenicity on soybean and sunflower was confirmed using a seed assay method (Manici et al., 1995), as well as the cut stem method (Twizeyimana et al., 2012). The same isolate (PPRI 23755) was used to inoculate both sunflower and soybean. The isolate was cultured on Potato Dextrose Agar (PDA, Difco) in the dark for seven days at 28°C. Fully grown colonies were cut into 0.5 cm^3 blocks, and 30 such blocks were added to each flask containing the sterile millet seeds. The flasks were incubated at room temperature (25°C to 30°C) for three weeks and shaken

daily. Following colonization of the millet seeds, the inoculum was air-dried in a laminar flow bench for ten days and stored at 4°C until planting.

2.2 Planting and growth conditions

The first experiment was planted in February 2017 and the second was planted in June 2017. Soybeans were harvested in May 2017 for the first experiment and September 2017 for the second, while sunflower was harvested in June 2017 and October 2017, respectively. Soil with no previous history of *M. phaseolina*, obtained from a farm in the Leandra area in Mpumalanga, was steam-sterilized prior to planting. The soil consisted of 69% sand, 17% silt and 14% clay. The bulk density of the soil was 1224.6 kg/m^3 with a pH of 5.6 (KCl). The cation exchange capacity of the soil was 5.1 cmol$_c$/kg. Pots (13 cm high, 15 cm top diameter and 11 cm bottom diameter) were filled three quarters with soil. The soil was drenched with sterile distilled water 24 hours before inoculation and planting. The field capacity of the soil was 38%. This was determined after drenching the soil of six pots with water and allowing them to drain for 24 hours, after which the moisture content was measured with a soil moisture meter from Selectech (Johannesburg, South Africa) (Fuhlbohm et al., 2013). The soybean cultivar PAN1500R and the sunflower cultivar PAN7080 from PANNAR Seed (Pty) Ltd. (Greytown, South Africa) were selected for the experiment as they are popular cultivars among South African producers. Although there is no recorded information on cultivar susceptibility to charcoal rot in South Africa, these two cultivars have shown susceptibility to charcoal rot. PAN1500R is a soybean plant with compact indeterminate growth habit in the maturity group 5.8. The sunflower cultivar PAN7080 is a medium-to-late cultivar.

One gram of *M. phaseolina* millet seed inoculum was added directly to the soil in each pot of the inoculated treatments and mixed into the upper 2 cm of the soil before planting on

the same day (1 g inoculum / 30 cm^2). This was determined to be sufficient for disease development in a pilot study (Jordaan, unpublished). The soybean inoculant *Bradyrhizobium japonicum* (strain WB74), known commercially as Stimuplant (RolfesAgri, Pretoria, South Africa), was added only to the soybean seeds as prescribed by the manufacturer. This is a standard practice in South African soybean production and aids in nitrogen fixation. Seeds of the soybean cultivar PAN1500R and the sunflower cultivar PAN7080 from PANNAR Seed (Pty) Ltd. (Greytown, South Africa) were sown to a depth of 2.5 cm. After a single set of leaves was fully formed, the plants were thinned out to three plants per pot. A soil moisture meter was used to measure the soil moisture content at weekly intervals. After emergence, plants in the RSM treatments were watered at the first sign of wilting (6.6% soil moisture) and only received half as much water (i.e. soil moisture fluctuating between wilting point and below field capacity) as the plants in the irrigated treatments (Fuhlbohm et al. 2013). All treatments not subjected to water stress were watered daily. Temperature, light intensity and humidity were monitored with the use of HOBO U12 Temp/RH/Light/External Data loggers (Onset, Cape Cod, Massachusetts) set to log every 30 minutes throughout the duration of the experiments in the greenhouse.

2.3 Experimental design and sampling time

A pot experiment was conducted under greenhouse conditions that included both soybean and sunflower. The pot experiment was repeated. Each experiment was laid out using a factorial randomized complete block design with seven replicates. Each replicate consisted of three pots, with three plants per pot. Four treatments were applied to both the soybean and sunflower crops. The four treatments included an inoculated and water stress treatment

(IRSM), an uninoculated and water stress treatment (URSM), an inoculated and irrigated treatment (II) and an uninoculated and irrigated treatment as a control (UI).

2.4 Disease evaluation

2.4.1. Soybean

At V5 (just following seedling stage), when five trifoliate leaves had developed (Fehr and Caviness, 1977), disease development was destructively assessed as described below on two replicates (18 individual plants). At flowering stage (R2), when the plants were in full bloom, disease evaluation was conducted on a further two replicates. Any lesions observed on the outside of the stems were also measured. The remaining three replicates were evaluated at maturity, when at least one pod per pot had reached its mature color (R7). At this stage the number of dead plants was recorded, lesions present were measured and the number of plants that retained their leaves was also recorded.

2.4.2. Sunflower

Two replicates were assessed for disease incidence, severity and colonization after seedling stage, at V4, when four true leaves were at least 4 cm long (Schneiter and Miller, 1981). Lesions on the outside of the stems were measured, and disease evaluations were again conducted on two replicates at flowering (R5 stage, coinciding with the emergence of ray flowers in sunflowers). At maturity, when the bracts started to turn brown (R9), the remaining three replicates were assessed. Lesions on the outside of the stems were again measured and the number of dead plants was recorded.

Disease incidence (%) was calculated as the percentage of plants per treatment showing discoloration within the stem. Severity was measured using a pictorial scale from 1 (no visible

microsclerotia) to 5 (almost complete discoloration due to microsclerotia of the lower stem and tap root sectioned longitudinally) (Mengistu et al., 2007). A disease index was calculated for each treatment as (incidence*severity)/(total number of observations made per treatment).

Pathogen colonization was determined for both soybean and sunflower following the adapted protocols from Almeida et al. (2003) and Anis et al. (2013). The stem between the cotyledons and the first root was sliced longitudinally. These pieces were rinsed in sterile distilled water to remove excess soil and placed in a 5% sodium hypochlorite solution for two minutes. Subsequent to being rinsed twice with sterile distilled water, 1 cm pieces were cut from the sterile stem section and labelled according to the position on the stem. Pieces were placed onto sterile tissue paper to dry before plating, in sequence, onto amended PDA medium (containing 100 mg/L Rifampicin and 0.5 ml/L Propamocarb) according to Mengistu et al. (2007). Plates were incubated at 28°C for three days in the dark. The segments yielding *M. phaseolina* growth were recorded.

2.5 Yield evaluation

2.5.1 Soybean

At maturity, the number of pods per plant was counted. Seeds were removed from the pods and weighed separately per plant. A total of 108 plants were evaluated for yield.

2.5.2. Sunflower

Seeds were removed from each sunflower head at maturity and counted. The seeds of each individual plant were weighed separately and recorded. A total of 108 plants were used for yield assessment.

2.6 Statistical analysis

Factorial data analyses were done using Statistical Analysis Software (SAS version 9.4). The main effects were inoculated and uninoculated (main effect one), irrigated and RSM (main effect 2) and stage of sampling (main effect three). Data were normally distributed with homogenous treatment variance according to the univariate procedure and normality plots. Analysis of variance (ANOVA) was used to test for differences between the four treatments for all data except for severity and colonization. Treatment means were separated using Fisher's Protected Least Significant Difference (LSD) at the 5% level of significance. Severity was tested, using ANOVA on ranks with Tukey's studentized range test for separation of the means, also at the 5% level of significance. Colonization data were analysed using Chi-square analysis. A 2x4 contingency table was used. The expected frequencies were calculated by taking the row total*column total/grand total (where rows were 0 cm, 1 cm, 2 cm and 3 cm, and columns were II and IRSM). The Chi-square value was compared to that of the Chi-square table to determine the significance of H_0. Yield loss due to charcoal rot was calculated as the URSM treatment yield less the IRSM treatment yield. The following criteria were used to determine if the effects of RSM and pathogen infection were independent, additive or synergistic on yield reduction, disease index and colonization, based on the treatment means:

Additive interaction: $A = B + C$

Synergistic interaction: $A > B + C$

Independent interaction: $A = B$

where A is the IRSM treatment mean; B is the II treatment mean and C is the URSM treatment mean.

3. Results

During the first pot experiment, the minimum temperature ranged from 20°C to 24°C and the maximum temperature from 24°C to 29°C. The second pot experiment had similar minimum (21°C to 26°C) and maximum (26°C to 31°C) temperatures. The average relative humidity was 56% for the first experiment and 37% for the second experiment. The average light intensity in the greenhouse for both experiments was 75 Lux, with average day lengths of 12 hours and 11.5 hours per day for the first and second experiments, respectively.

Both crops were adequately stressed during both experiments, as was evident from the harvested seed weight in the RSM treatments compared to the optimally irrigated treatments. Analysis of variance at 95% significance did not show any significant differences in disease index, severity or colonization between the first and second experiments for either soybean or sunflower. Disease index, severity, incidence, colonization and yield data for the first and second experiments were pooled for both crops.

3.1 Soybean

Spindle-shaped lesions were only observed on the surface of soybean stems of inoculated plants in the RSM treatments. Microsclerotia were visible on the surface of the stems at maturity only after irrigation was terminated. At maturity, only 41% of the IRSM soybean plants and 43% of the URSM soybean plants retained their leaves, and it was apparent that defoliation was due to RSM conditions. Plants within the IRSM treatments were more susceptible to early senescence compared to the other treatments ($p=0.032$).

Even though the isolate used in the experiment, PPRI 23755, was originally isolated from sunflower, the disease index was 20% higher in the soybean plants than in the sunflower

plants grown under the same conditions in the greenhouse (p=0.042), as shown in Figure 1 and 3. A synergistic interaction between RSM and infection was observed (p=0.019) as the disease index was higher in the IRSM than in the URSM and II treatments. Disease incidence and severity were higher at maturity than at the seedling or flowering stages (p=0.028). Interaction between the soil moisture content, infection by the pathogen and the plant stage had the biggest influence on disease severity (p=0.027) and incidence (p=0.03). RSM and infection were synergistic for incidence but independent for severity. Consequently, disease index was affected by the interaction between RSM, the presence of the pathogen and the growth stage of the crop (p=0.013).

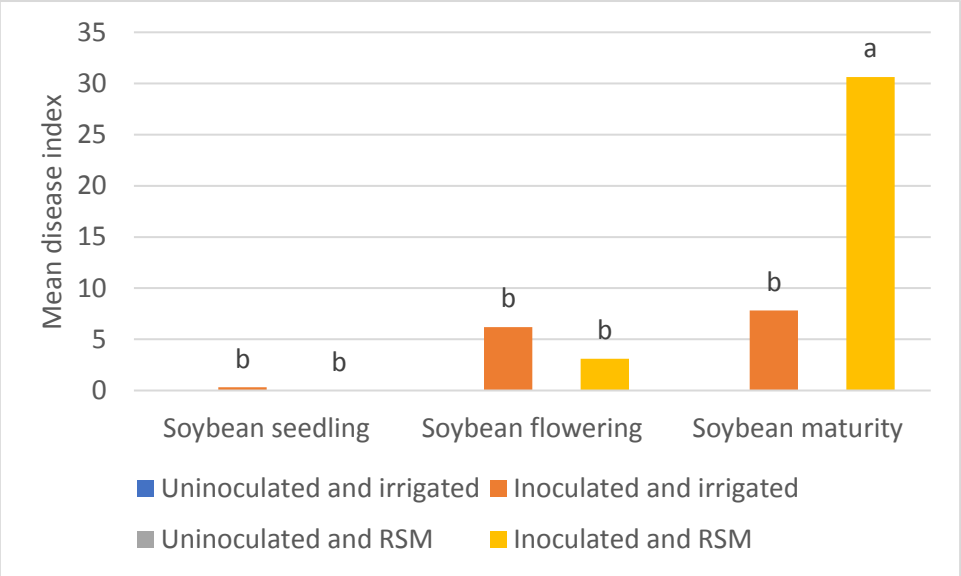

Fig. 1: Mean charcoal rot index in soybeans at seedling stage (V5), flowering stage (R2) and at maturity (R7) for both experiments pooled (p=0.042).

Soybean stems at 1 cm and higher were colonized 10% less frequently by *M. phaseolina* than sunflower stems. The pathogen was not isolated past the first internode in either the RSM or the irrigated treatments. RSM only had a significant effect on colonization of the stems at maturity (Figure 2 a, b). The most soybean stems were colonized at maturity in the IRSM treatment, $\chi^2(3, N=216)=16.49$, p=0.01. Colonization frequency was lower under irrigated

conditions than in the plants subjected to RSM. The presence of the pathogen in the soil and RSM independently influenced colonization, irrespective of growth stage.

The *M. phaseolina* isolate PPRI 23755 contributed to a yield loss of 6.13% in soybean compared to the URSM treatment. In the inoculated optimal moisture treatments, a yield reduction of 12.7% in soybeans was observed when compared to the uninoculated and optimal moisture control treatment. Yield and pod count per plant were both significantly reduced in the RSM treatments (p=0.001) compared to the irrigated treatments (p=0.323).

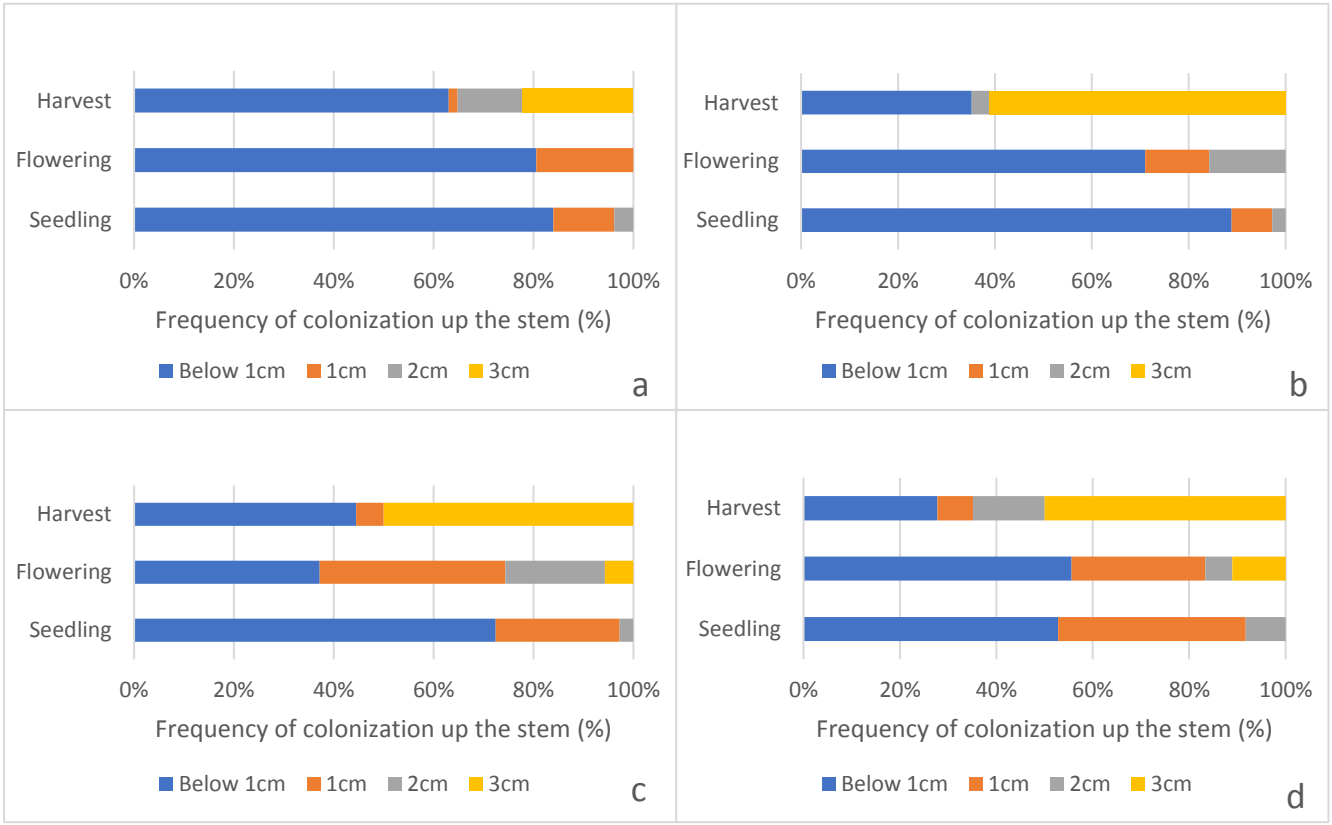

Fig. 2: Frequency of colonization within the inoculated irrigated soybean (a), inoculated reduced soil moisture soybean (b), inoculated irrigated sunflower (c) and inoculated reduced soil moisture sunflower (d) treatments at seedling, flowering and maturity stages.

3.2 Sunflower

At maturity, after irrigation was terminated, microsclerotia were visible on the surface of the stems. Lodging was noted in some of the sunflowers, but this was attributed to thin stems of individual plants grown in the pots and not RSM or inoculation. Early senescence of sunflowers was more frequent during the second experiment than the first, in the RSM treatments (irrespective of inoculation) closer to maturity (p=0.023).

Disease index was 20% lower in sunflower (p=0.042) than in soybean, as shown in Figure 1 and Figure 3. Disease index was higher in the IRSM than in the URSM and II treatments, suggesting a synergistic interaction between RSM and infection (p=0.019). Higher disease incidence and severity were more closely associated with the maturity stage than the seedling or flowering stages (p=0.028). Incidence and severity in sunflowers were mostly increased by RSM (p=0.053). Disease index was affected by the interaction between RSM, the presence of the pathogen and the growth stage of the crop (p=0.013).

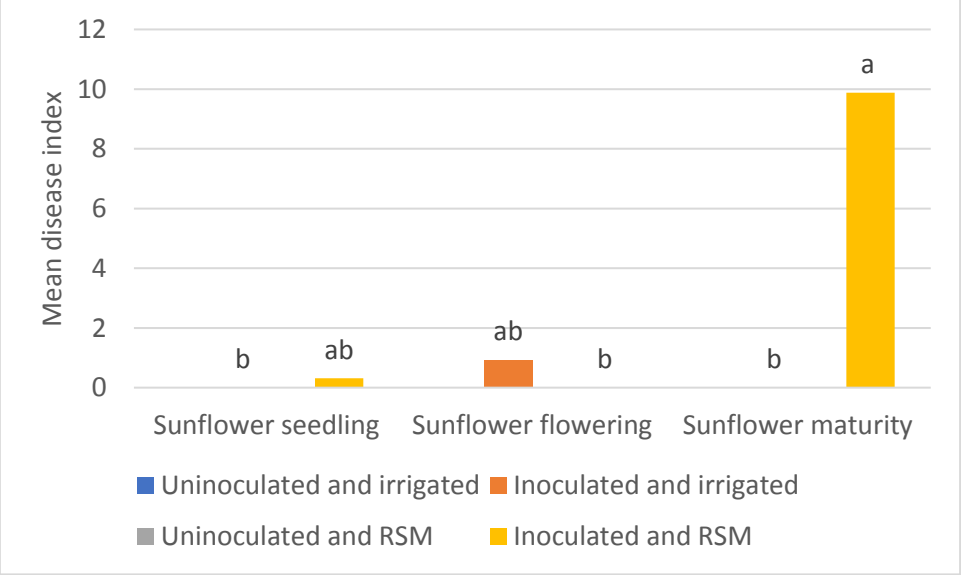

Fig. 3: Mean charcoal rot index in sunflower at seedling (V4) stage, flowering (R5) stage and at maturity (R9) for both experiments pooled (p=0.042).

Macrophomina phaseolina was isolated 10% more frequently from sunflower than from soybean stems at 1 cm and higher, and not past the first internode. At maturity, RSM had a significant effect on stem colonization (Figure 2 c, d). Colonization observed at maturity and in the IRSM treatment was also higher than at flowering or seedling stage, or in the II treatments $\chi^2(3, N=216)=33.22$, $p=0.01$. Frequency of colonization was higher under RSM conditions than in the irrigated plants. The presence of the pathogen in the soil and RSM were independent variables for colonization throughout the cropping season.

Charcoal rot contributed to 12% yield loss in sunflower compared to the URSM treatment. Sunflower yield in the inoculated and optimal soil moisture treatments was 8.2% higher than in the uninoculated and irrigated treatments. Overall, the sunflower yield and number of seeds per head were significantly more affected by RSM ($p=0.001$) than by the IRSM treatment ($p=0.558$).

4. Discussion

There have only been a few reports of charcoal rot in South Africa to date (Craven, 2016; Knox-Davies, 1965; Phillips, 2014; Smit and Knox-Davies, 1989). The increase in daily maximum temperatures and the decrease in rainfall in the country expected due to climate change (Van der Waals et al., 2013; Ziervogel et al., 2014) could result in this disease becoming the cause of economic loss in the oilseed industry. The main drivers of charcoal rot are infection of the host by *M. phaseolina* coupled with conducive environmental conditions such as RSM. The current study indicated that adequate soil moisture does not delay infection of the host tissue at seedling stage, and that colonization of the stem is favoured by RSM at maturity.

A symptom often associated with charcoal rot in soybeans is retaining of the leaves at maturity (Luna et al., 2017; Mengistu et al., 2007). However, at the end of the season, in these

pot experiments, only 41% of inoculated plants (under RSM conditions) had retained their leaves, which corresponds with the report of Almeida et al. (2003). In the current study, the presence of the pathogen did not seem to influence defoliation, indicating that RSM was the primary cause of defoliation.

At seedling stage, in both the II and IRSM treatments, the pathogen was most frequently isolated from the first centimetre above the root zone, while at maturity, the majority of isolations were from higher up in the stems. The pathogen gradually colonizes the stem as the season progresses. This was also found by Kendig et al. (2000), where the most microsclerotia in soybean stems were observed at the R8 stage, compared to R2, R4, R5 and R6. Early infection, at seedling stage, can result in greater colonization up into the stem of the host and in turn greater yield loss compared to when infection only starts at flowering stage. Pearson et al. (1984) related the number of colony-forming units per gram of host tissue at maturity to yield and found that as the population density increased, yield decreased. The timing of *M. phaseolina* infection in a given host differs, as was shown to be the case for susceptible and tolerant sesame cultivars (Chowdhury et al., 2014) as well as for different soybean cultivars (Kendig et al., 2000; Pearson et al., 1984). This demonstrated that the time of infection for optimal disease development and subsequent impact on yield should be investigated. However, the pathogen was isolated more frequently from the IRSM compared with the II plants, which indicates that RSM and crop stage are important for colonization into the stem tissues (Dodd, 1980). Pearson et al. (1984) and Kendig et al. (2000) suggested that different soybean cultivars might also vary in their ability to be colonized and that once within the roots, the pathogen population increases at different rates in different cultivars. *M. phaseolina* was more frequently isolated from sunflower stems than soybean stems, suggesting differential host responses. However, the final disease index on soybean was significantly higher than on sunflower, indicating that the *M. phaseolina* isolate produced microsclerotia more readily on soybean than

on sunflower. Incidence and severity ratings were based on the visual detection of microsclerotia within the stem epidermal and vascular tissue and did not take latent infection into consideration.

Almeida et al. (2008) investigated different crops grown in rotation with one another and the resulting microsclerotia per gram of root that were produced at the end of the growing season. Soybean had the highest number of microsclerotia per gram of root, while sunflower had the least (Almeida et al., 2008), similar to our findings. Higher microsclerotia densities on soybeans could lead to a greater increase in inoculum in the soil when soybeans become infected by *M. phaseolina* compared to sunflowers. This has implications for crop rotation, in particular in the choice of crop that follows soybean. Microsclerotia were only visible on the surface of stems at maturity after irrigation was terminated in this study. This is similar to the findings of Almeida et al. (2003), Kendig et al. (2000) and Pearson et al. (1984), where microsclerotia could only be seen after the R7 stage in soybean.

The pathogen and growth stage interaction was highly significant on disease incidence and severity, as was the pathogen and RSM interaction. When considering root infections, Kendig et al. (2000) found a significant interaction between soil moisture content and growth stage, similar to this study where infection rate increased towards maturity. Disease incidence in inoculated soybean and sunflower plants grown under RSM conditions revealed a synergistic effect between RSM and infection by the pathogen. Although low soil moisture content is usually associated with increased infection and charcoal rot, this is not always a requirement (Almeida et al., 2003), as confirmed by the results of this experiment. Other research has also shown that infection can take place within six weeks of planting, irrespective of soil moisture content (Bruton et al., 1987; Kendig et al., 2000).

The effect of RSM and the presence of the pathogen in the plant is synergistic to yield reduction, for both soybean and sunflower, based on the formulae proposed in this study. Most studies agree that, as the number of colony-forming units increases, the yields tend to decrease, and flowering stage has been identified as the critical time for infection that will result in yield loss (Mengistu et al., 2011b, Pearson et al., 1984; Short et al., 1980). Yield reduction in this experiment was predominantly a result of RSM, as there was no significant difference in yield between the URSM and IRSM treatments. Kendig et al. (2000) also found that irrigation alone had a significant effect on yield, and attributed this to the late infection of the host. Sclerotial viability in the soil could lead to decreased infection rates and explain a decrease in stem colonization resulting in lower than expected yield loss. Temperature and soil moisture affect germination of sclerotia (Luna et al., 2017). Papavizas (1977) found a decrease in sclerotial viability at 15% and 30% soil moisture-holding capacity. During both trials, reduced soil moisture treatments commenced soon after emergence and could have contributed to reduced sclerotial germination. This emphasises the timing of drought conditions only after initial germination and infection of the host.

Another reason for lack of yield loss in soybeans could be the addition of Rhizobium. Some Rhizobium from Pakistan and arbuscular mycorrhiza fungi have been seen to reduce *M. phaseolina* growth *in vitro* (Gupta et al., 2012). Chakraborty and Purkayastha (1984) demonstrated that rhizobitoxine produced by *Bradyrhizobium japonicum* reduces mycelial growth of *M. phaseolina* in culture.

When comparing the yield loss in both the inoculated and non-inoculated RSM treatments, charcoal rot accounted for 6.13% yield loss in soybean and 12% in sunflower. Mengistu et al. (2011b) reported an additional 7% yield loss attributed to RSM alone when comparing RSM field trials of artificially inoculated and non-inoculated plots. This also indicates a synergistic effect of RSM and charcoal rot on yield loss. The soybean yield of the

II treatment was decreased even under optimal soil moisture conditions. Mengistu et al. (2011a) also reported yield reduction in susceptible soybean isolates under conditions suboptimal for charcoal rot development. An increase in yield, however, was observed in this study in the inoculated sunflower plants grown under optimal moisture conditions. Fungi producing plant growth hormones such as auxin are not uncommon, and other pathogenic fungi, such as *Fusarium*, have been known to promote plant growth (Bitas et al., 2015; Chanclud and Morel, 2016). Why this is the case for sunflower and not for soybean is not clear.

Colonization and disease index showed that the environmental conditions in the greenhouse were optimal for infection and disease development, especially in soybeans. However, when considering the lack of typical charcoal rot symptoms and the fact that yield reduction due to *M. phaseolina* was low, the following aspects of this disease seem to be important: (i) timing of infection, (ii) possible inoculum threshold, and (iii) virulence of South African isolates including the possible effect of multiple *M. phaseolina* isolate infections. A better understanding of disease development could be used to improve disease management through disease escape, either through the use of irrigation at critical times during the season, or the use of cultivars where flowering coincides with cooler temperatures.

5. Conclusion

Results from this study support findings that irrigation will not prevent infection of soybeans by *M. phaseolina* but may significantly reduce disease severity in infected plants. Selective host preference was observed in this sunflower isolate towards sunflower plants, resulting in a slightly higher yield loss under RSM conditions; however, microsclerotia were more concentrated in soybeans, which might consequently increase subsequent soil inoculum. Resistance screening should therefore rely not only on isolation of the pathogen from the host

but also on yield assessments. Charcoal rot symptoms on soybean and sunflower were only apparent at maturity, and because charcoal rot coincides with drought conditions, yield loss due to the disease often goes unnoticed by South African producers. Future research should include determination of the drivers behind time of infection within the growing season and how this might influence disease development. The effect of different soil inoculum concentrations on the rate of tissue colonization and subsequent yield reduction should be investigated further. Knowledge about other environmental or biological contributors to the disease, such as the response of different cultivars to the pathogen, infection of the host with multiple *M. phaseolina* isolates and the contribution to disease development by co-infections with other pathogens, will improve the current understanding of this disease. Possible sources and dissemination of secondary inoculum should also be investigated. Results from this study show that RSM conditions are not required for infection, but rather colonization of the host, and that yield reduction due to *M. phaseolina* infection might require higher colonization densities or other environmental factors. In addition, incidence and severity of charcoal rot are not only affected by RSM but also the growth stage of the host.

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