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In-field climatic factors driving Sclerotinia head rot progression across different sunflower planting dates

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Abstract

Sclerotinia head rot, caused by *Sclerotinia sclerotiorum*, is a major disease limiting sunflower production in tropical and subtropical agroecological zones. Sporadic outbreaks across South Africa have resulted in major losses, yet little is known about the in-field climatic factors driving this infection. Short-interval, staggered plantings have been proposed as a control method for Sclerotinia head rot, which help to limit the number of plants in a susceptible developmental stage during conducive environmental conditions. However, this complicates field management practices, especially if working at the fringes of a planting window due to delayed rains. This study aimed to investigate the effect of planting date on Sclerotinia head rot progression in monthly plantings across the summer period. Artificial mycelial plug inoculations were performed at the R5.9 flowering stage in an open field. Disease establishment, progression and severity were monitored at 3-day intervals for 30 days. We show that disease establishment was delayed by low relative humidity or extreme low temperatures in the January and March planting dates where the first lesions were only observed 6 days post-inoculation. Consistently high temperatures above 27°C also suppressed disease progression and produced low area under the disease progress curve (AUDPC) scores of 75.15 and 29.4 for the October and November planting dates, respectively. These findings suggest that regardless of season or location, selecting a planting date that ensures the sunflower bloom period aligns with the hottest, driest part of the season will probably suppress Sclerotinia head rot in regions with average summer highs above 27°C.

KEYWORDS
average rainfall, average temperature, *Helianthus annuus*, late planting dates, relative humidity, white mould

1 | INTRODUCTION

Sunflower (*Helianthus annuus*) is the Republic of South Africa's (RSA) third most important crop (after maize and soybean), where the RSA is a major sunflower-producing country in south-eastern Africa (Pilorgé, 2020) and ranks 15th globally (FAOSTAT, 2022). Sunflower is mostly grown under dryland conditions in the summer rainfall regions of the country and is usually planted from November to January (Anonymous, 2009). Sunflower can also be considered a catch crop due to its hardy nature and is often grown as an alternative to soy or maize in dry years when rains are delayed (Meyer & van der Burgh, 2015). Local production has been declining since 1999, particularly in the North West province, due to several factors including *Sclerotinia* disease incidence (Meyer & van der Burgh, 2015). Sclerotinia head rot is the most significant yield-limiting disease in sunflower, and *Sclerotinia sclerotiorum* was identified as the causal agent (Bolton et al., 2006).

Sclerotinia head rot has been a disease of major concern in RSA since the first severe outbreaks in the late 1970s (Holtzhausen & Van der Westhuizen, 1980). During the RSA 2013/2014 growing season, Sclerotinia head rot caused sunflower losses of up to 60% due to the cool, wet conditions (Crave et al., 2016). In the 2019/2020 season, sunflower planted in Clocolan (Free State, RSA) on 19 December 2019 had around 50% Sclerotinia head rot incidence, whereas the January plantings (16 and 27 January 2020) had lower incidences of only about 3% and 6%, respectively (Meiring et al., 2021). It was recently reported in *Farmers Weekly* that Sclerotinia head rot disease incidences of up to 90% were observed on late-planted sunflowers that mature under cool and wet conditions in the North West and northern Free State provinces (Flett, 2022). These reports suggest that those environments associated with a specific planting date are important drivers of Sclerotinia head rot establishment and progression; however, no studies have assessed this in RSA or globally.

Limited measures are currently available to control Sclerotinia head rot. Agricultural production practices, such as crop rotations, are ineffective due to the wide host range of *S. sclerotiorum* and the long survival time of soilborne sclerotia (Rothmann & McLaren, 2018). Short-interval, staggered plantings have been suggested as an escape strategy to limit the number of plants flowering during periods of favourable climatic conditions for Sclerotinia head rot development. However, in seasons with late rain onset this may be difficult to manage, especially around the end of the planting window (Bureau for Food and Agricultural Policy, 2020). Only two biological or chemical control agents against *S. sclerotiorum* are registered in RSA: the fungal biological control *Coniothyrum minitans* and the fungicide benomyl for preventive use on sunflower seed (Rothmann & McLaren, 2018). The use of benomyl is prohibited in the United States and Europe due to its toxic effects and is regarded as a food and environmental contaminant (Mehtap et al., 2021), thus suggesting a limited life span for this chemical in RSA. Furthermore, some studies have identified *S. sclerotiorum* strains that are resistant to benomyl, which may further limit the usefulness of this chemical as the pathogen evolves (Gossen et al., 2001). Currently, integrated disease management strategies remain the primary tool to combat infection in sunflower, but the continued unpredictable and severe outbreaks of Sclerotinia head rot suggest that the current practices still require adjustments.

S. sclerotiorum is one of two very similar *Sclerotinia* species that have been identified in RSA (van der Westhuizen & Eicker, 1988). This necrotrophic fungus is prevalent in humid and temperate sunflower-growing regions globally (Bolton et al., 2006). *S. sclerotiorum* has a wide host range of more than 400 host species (Bolton et al., 2006) and includes a number of agriculturally important field and vegetable crops (Rothmann & McLaren, 2018). The pathogen relies on sclerotia (a melanized mycelial mass) for long-term survival in the soil (Bolton et al., 2006). *Sclerotinia minor* is the other *Sclerotinia* species that has been reported in RSA and has similar growth conditions and host ranges (Melzer et al., 1997). *S. sclerotiorum* and *S. minor* have almost indistinguishable morphological traits, with subtle differences in the size and texture of sclerotia, with side-by-side comparisons of these structures being used to identify the species in the past (Laemmlen, 2002). With the advent of molecular tools and markers, more accurate sequence or PCR confirmations can now be performed (Abd-Elmagid et al., 2013).

Sclerotia are the principal infectious propagules for *S. sclerotiorum* and can germinate either carpogenically or myceliogenically (Bolton et al., 2006). Carpogenic germination produces sexual fruiting bodies (apothecia). Apothecia formation generally requires temperatures between 5 and 25°C, with temperatures above 26°C being detrimental to apothecia production and survival (Smolińska & Kowalska, 2018). Ascospores are wind-dispersible and can lead to the colonization of different above-ground plant tissues (Bolton et al., 2006). In sunflower, head rot results from carpogenic germination of ascospores that tends to occur under 85% relative humidity and temperatures between 5 and 25°C (Clarkson et al., 2014; Huang & Kozub, 1991). The ascospores germinate on the sunflower head and enter the plant tissue through senescing florets, resulting in disease establishment (Bečka et al., 2016; Harikrishnan & Rio, 2008; Payen, 1983). Once infection has been established, necrotic lesions form on the back of the sunflower head (Gulya et al., 1997). Infection spreads throughout the head, disintegrating and shredding the soft tissue to the typical broom-like appearance. Sclerotia develop in the head after 7–10 days and drop to the ground to re-establish infection (Gulya et al., 1997).

Though extensive research into the lifecycle of *S. sclerotiorum* has been done, there have been far fewer studies focused on understanding the in-field environmental factors facilitating disease establishment and progression in the sunflower head during a Sclerotinia head rot infection. Studies by Bester (2018) and Meiring et al. (2021) were carried out in RSA where sclerotia applied to the soil or ascospore suspensions were applied at different flowering stages to investigate the disease epidemiology. These two studies showed Sclerotinia head rot incidences in

natural field conditions to be between 40.4% and 53.3%. They assessed disease incidence across several planting dates including a November planting date, four December planting dates and a January planting date, but did not assess the whole growing season or how environmental factors influenced disease progression within the sunflower head.

In the current study, we aimed to investigate the influence of climate factors associated with monthly planting dates on Sclerotinia head rot progression across the entire growing season. The objectives of this study were (a) to artificially inoculate plants sown at different monthly planting dates with *S. sclerotiorum* mycelia at a late flowering stage (Figure 1) and monitor disease

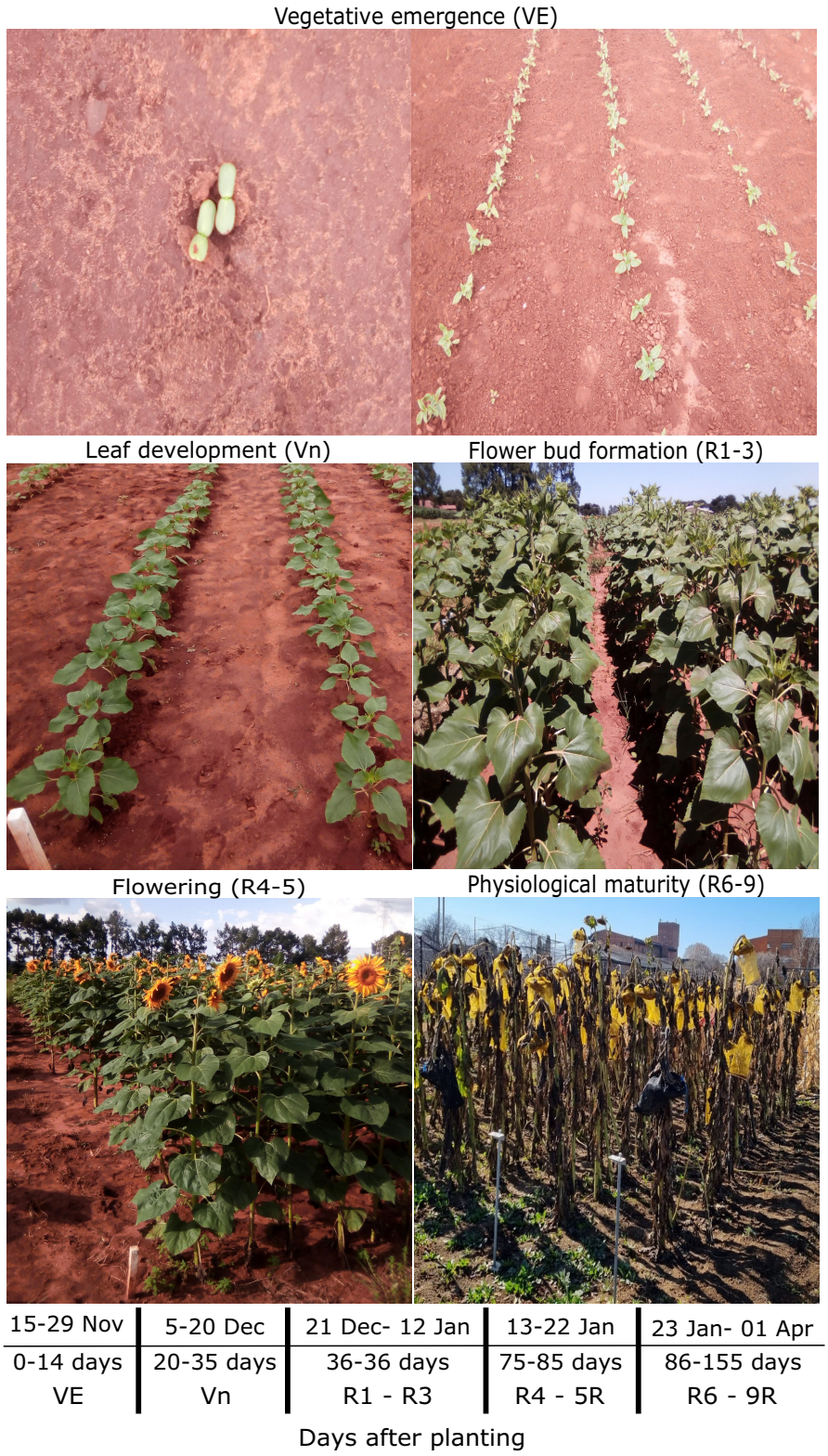

FIGURE 1 A general sunflower growth calendar for a medium-long maturing cultivar (e.g., PAN 7080, used in this study) grown in the Republic of South Africa. Images indicate the different growth stages and the names of each stage (VE, Vn, R). The schematic at the bottom indicates the optimal planting date and general dates for each growth stage. The duration in days of each growth stage is indicated as number of days after planting with a total of approximately 155 days to reach maturity. [Colour figure can be viewed at wileyonlinelibrary.com]

establishment and progression in the head; (b) to correlate disease establishment, progression and severity with prevailing climate factors such as rainfall, temperature and relative humidity over the inoculation and monitoring period; and (c) to evaluate disease severity across the six planting dates over this period. According to our knowledge, this is one of the first studies to detail disease progression within the sunflower head at different planting dates in the field and correlate this with climatic conditions during infection and disease progression. We found strong correlations between planting date and Sclerotinia head rot progression that were primarily driven by temperature at the time of infection. Our findings suggest that sunflower plantings that align flowering with the warmer parts of the season would be beneficial in limiting Sclerotinia head rot damage. However, the close association with climate at the time of infection suggests there is unlikely to be a single planting date that consistently limits Sclerotinia head rot due to high seasonal variability.

2 | METHODS AND MATERIALS

2.1 | Plant material and planting date trials

In RSA, the sunflower planting window runs from November to mid-January. The sunflower hybrid PAN7080 (Corteva) was selected for this study as the cultivar is widely planted across RSA and has a medium to late maturity (Figure 1), with a moderate tolerance to Sclerotinia head rot (Pannar, 2023). PAN7080 was planted in an open field (25°45′ S, 28°16′ E) at the Innovation Africa campus (University of Pretoria, Gauteng Province, South Africa) located 1327 m a.s.l. Pretoria has a humid subtropical climate with summer rains, an average annual rainfall of 706 mm (Köppen et al., 2011), an average summer maximum temperature of 26.7°C and minimum temperature of 13.9°C (http://www.pretoria.climatemps.com/). The trial was duplicated at the Agricultural Research Council (ARC), Grain Crops Institute in Potchefstroom (−26.7294, 27.0823) as part of a larger study for purely observational assessments of disease. The Potchefstroom site is 1352 m a.s.l. (Baier, 1966). Potchefstroom has a cold semi-arid:steppe climate with summer rains and annual rainfall averaging more than 600 mm (Köppen et al., 2011). Mean monthly maximum summer temperatures are over 32°C, while the mean monthly minimum temperatures fall below −1°C (South African Weather Bureau, 1954). The artificial inoculation trial for Sclerotinia head rot progression was conducted only in Pretoria; however, Sclerotinia head rot was observed at both sites (Table S1).

For the Pretoria inoculation trial, seeds were sown in a split-plot design with three replicates across six monthly planting dates from spring to autumn (Table S2). Inter-row spacing of 90 cm and in-row spacing of 25 cm were used. At the time of sowing, 70 kg/ha nitrogen (LAN, Sasol South Africa [PVT] Ltd) and 21 kg/ha phosphorus (Petrow Agri) were applied, followed by top dressing with 100 kg/ha nitrogen (LAN) 28 days after planting. The field was maintained by manual weeding with no pest or disease control measures implemented during the trial. The trial was rainfed, with no supplementary irrigation.

2.2 | Weather data

Relative humidity, precipitation, minimum temperatures and maximum temperatures were selected as variables for this study to be in line with previous growth factors established for *S. sclerotiorum* infection (Bolton et al., 2006; Clarkson et al., 2014). The data were collected from a manual weather station situated about 600 m from the trial plots for the period 15 January 2022 to 27 July 2022. This corresponded with the start of the artificial inoculation of the earliest planting date and ended with the last day of disease monitoring for the last planting date. The daily minimum and maximum relative humidity and minimum and maximum temperature were averaged across 3-day intervals and a 3-day bin for total rainfall was calculated to align with the scoring days (Table S3).

2.3 | *S. sclerotiorum* culture processing

This inoculation trial falls within a greater planting date study, which was conducted at two sites (Pretoria, Gauteng and Potchefstroom, North West provinces of RSA) over two seasons (2020/2021 and 2021/2022). During these trials, each planting date at each site was monitored for Sclerotinia head rot occurrences (Table S1). Four samples of diseased plant material of the November planting for the 2020/2021 season at the Pretoria site were collected and stored at room temperature in brown paper bags until processing. Sclerotia were collected from the diseased plant material (Figure 2a) and surface sterilized using 80% ethanol (Merck) for 1 min. Sterilized sclerotia were rinsed three times in sterile distilled water (Figure 2b), aseptically cut into smaller pieces and placed onto full-strength potato dextrose agar (PDA, 15 g agar, 20 g dextrose, 4 g potato extract; Merck). Samples were incubated for 10 days at 25°C (Figure 2c). A pure culture was produced and used for inoculation trials. The culture was added to the culture collection of Innovation Africa at the University of Pretoria (accession number CN15D1).

Mycelia were harvested for molecular species confirmation. DNA was extracted using the Murray and Thompson (1980) protocol with modifications (File S1). To confirm the identity of the isolate as *S. sclerotiorum*, three molecular regions were amplified by PCR. The rDNA internal transcribed spacer (ITS) region was targeted for sequence confirmation of species identification (White et al., 1990). Additionally, species-specific primers were used to distinguish between *S. minor* and *S. sclerotiorum*. *S. minor* primers targeted the laccase 2 (*lac*) gene and *S. sclerotiorum*-specific primers targeted the aspartyl protease (*aspr*) gene (Abd-Elmagid et al., 2013). For primer sequences and details of the protocol,

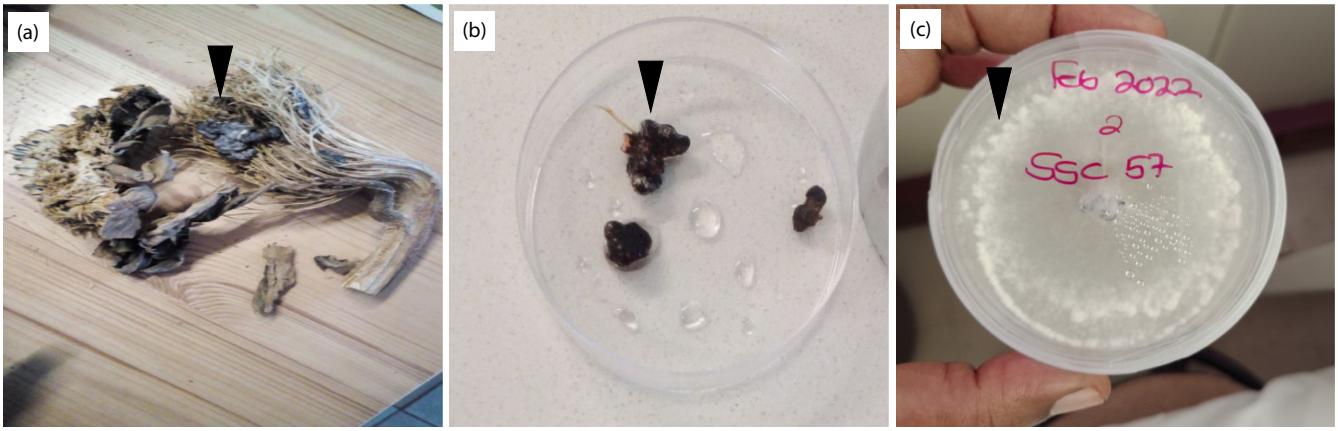

FIGURE 2 Inoculum preparation from sclerotia harvested at the Pretoria site in the first season (2020/2021). Sclerotia harvested from sunflower head with typical broom-like shredded appearance (a), with arrowhead indicating visible sclerotia. Sclerotia harvested from the head were surface sterilized in 80% ethanol and sodium hypochlorite (b), arrowhead indicates sterilized sclerotia. After plating sclerotia on potato dextrose agar, typical white *Sclerotinia sclerotiorum* mycelia (black arrowhead) are visible (c) and can be subcultured as needed. [Colour figure can be viewed at wileyonlinelibrary.com]

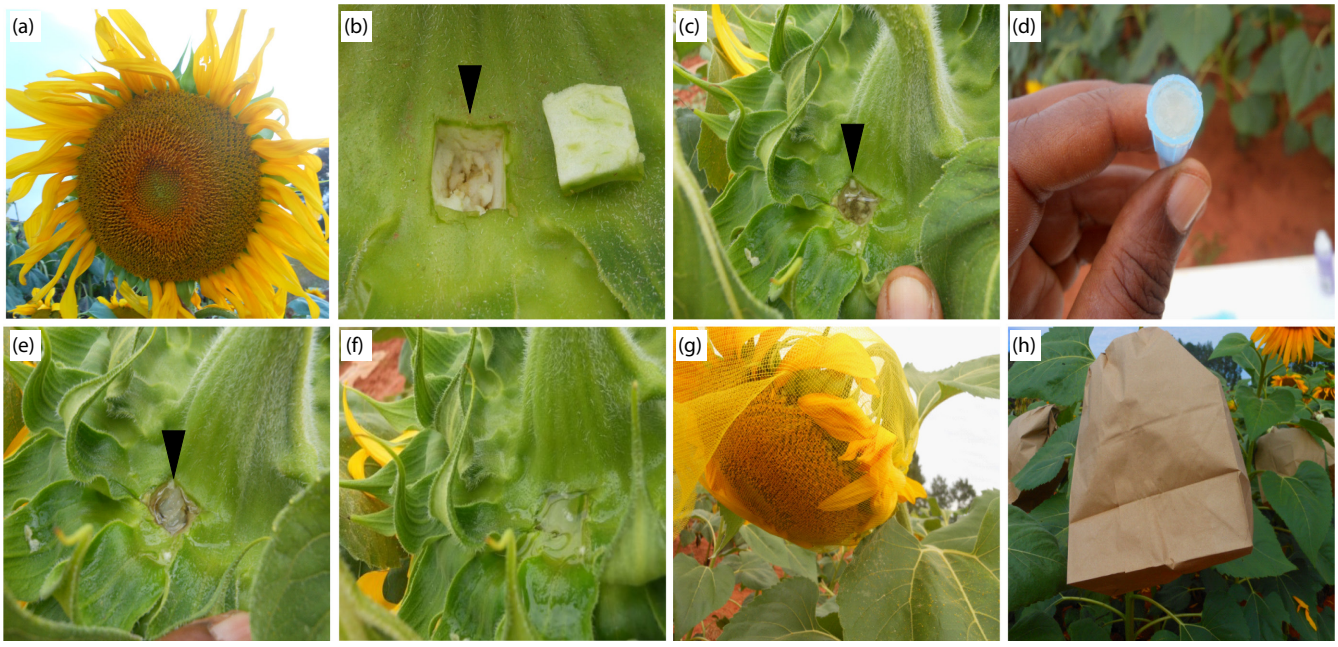

FIGURE 3 Mycelial plug method of inoculation to ensure infection and vigorous growth so that disease progression in the head can be monitored. Plants at the R5.9 growth stage (a) were selected for inoculation (Schneiter & Miller, 1981). A 1 cm^3 hole (b) was cut into the back of capitula (black arrowhead) for inoculation. The cut section was moistened with a rainwater spray (c). A 6-mm plug with mycelia obtained using the back of a sterile 1 mL pipette tip to cut out a plug from the actively growing mycelia in a standardized fashion (d). Mycelial plug is placed in the cut hole (e) of the capitula and sprayed with water again (black arrowhead). The removed piece of flower head is replaced to cover mycelial plug and again sprayed to ensure good moisture and disease establishment (f). The flower head is covered by a pollination bag (test plants; to limit bird damage) (g) or brown paper bags (positive control; to retain humidity) (h) and disease progression monitored. [Colour figure can be viewed at wileyonlinelibrary.com]

please see File S1. Fragments were amplified using standard PCR protocols as outlined in File S1.

All ITS amplicons were purified using Sephadex G50 columns (Sigma-Aldrich) and sequenced bidirectionally with the original primers. Sequencing was done with the BigDye Terminator Cycle Sequencing Kit v. 3.1 (Life Technologies) on an ABI 3500xL Genetic analyser (Applied Biosystems, ThermoFisher Scientific) at the DNA Sanger Sequencing facility (Faculty of Natural and Agricultural Science, University of Pretoria, RSA). The sequences were used as queries in a Basic Local Alignment Search Tool (BLAST) against the National Center for Biotechnology Information (NCBI) nucleotide database (Benson et al., 2012) prior to confirmation via phylogenetic analysis.

At the end of the trial, heads from four inoculated plants per planting date were collected for pathogen reisolation and reconfirmation of *S. sclerotiorum* as the causal agent in this trial. Sclerotia were harvested and cultures produced as described above. Mycelia were harvested from each culture for DNA extraction and PCR amplification of the three DNA regions for confirmation. Two samples per planting date were sent for Sanger sequencing. In all cases, DNA from *S. sclerotiorum* isolate 1980 (representative strain), *S. minor* strain CBS 339.39 (representative strain) and the original culture CN15D1 were included as controls (Wingfield et al., 2022). All ITS amplicons were sequenced and used in a phylogenetic analysis. All curated sequences were first aligned with the online version of MAFFT (https://mafft.cbrc.jp/alignment/software/; Katoh & Standley, 2013). A maximum-likelihood phylogeny, with *S. trifoliorum* as an outgroup, was generated using RAxML v. 8.2.1 (Stamatakis, 2014) with nonparametric bootstrap analyses of 1000 replications. The general time reversible (GTR) model (Tavaré, 1986) was employed with parameter optimization.

2.4 | Sunflower head inoculation

Isolate CN15D1 was used to develop the inoculum for the artificial inoculations. The culture was maintained by transferring a mycelia-covered PDA block from the growing edge of a plate to a new PDA plate every 3 months. Plates were sealed with Parafilm and incubated in the dark at 25°C for 84 h. This was repeated to bulk up mycelium for inoculations. Two different inoculation methods were used for the first rounds of inoculation. In the October and November planting date trials, the mycelial spray method was tested in the positive control inoculations, because it was reported to be closer to a natural infection (Bester, 2018), while the mycelial plug method is more robust (Chen & Wang, 2005). For spray inoculation, a mycelial suspension was prepared by suspending mycelia-covered blocks in rainwater, after which the suspension was sprayed onto the front and back of the sunflower head. However, the spray inoculation for the positive controls of the October and November sets was unsuccessful and so the mycelial plug method was used for the remainder of the experiment.

Twenty plants at the R5.9 flowering stage were randomly selected for inoculation from the three planting replicates of each planting date (Table S2), with border rows excluded from selection (Schneiter & Miller, 1981). Of these 20 plants, 10 were used as test replicates for the study group, while five plants each were used for the positive and negative controls (Table S2). Study and positive control plants were inoculated using the mycelial plug method (Chen & Wang, 2005). In short, a hole of about 1 cm^3 (Figure 3) was cut into the back of the head with a sterile surgical blade. The hole was sprayed with impurity-free rainwater, obtained from a collection tank, to increase humidity before a 7 mm-diameter disc of mycelia-covered PDA was cut with the back of a 1 mL pipette tip from the growing edge of the culture and placed, mycelia first, into the wound (Figure 3d,e). The plant tissue cut out was replaced on top of the PDA plug and sprayed again with rainwater (Figure 3f). Negative controls were mock-inoculated with sterile PDA blocks. All the heads were covered with pollination bags (study/test and negative control plants) to limit bird damage while still maintaining natural airflow (Figure 3g). Positive control plants were covered with brown paper bags to maintain high humidity conditions (Figure 3h) to ensure a conducive environment for disease.

2.5 | Disease scoring

Disease scoring was performed every 3 days from date of inoculation to 30 days post-inoculation (dpi) when heads were completely infected. The scoring interval was selected based on previous studies that showed successful infection through ascospores at 30–40 h or 48–72 h post-inoculation (Abawi & Grogan, 1975; Payen, 1983; Ratkos & Nagy, 1992). Disease severity was scored based on the area of the head infected. A scale of 0–5 was used (Figure 4a–f), where 0 = no observable symptoms, 1 = <12.5% infection, 2 = 12.5%–25% infection, 3 = 26%–50% infection, 4 = 51%–90% infection and 5 = >90% of the head infected. Disease severity scores were recorded and disease severity index (DSI) or area under the disease progress curve (AUDPC) calculated (Madden et al., 2007; Van Becelaere & Miller, 2004). The Shaner and Finney (1977) AUDPC equation was used in this study:

$$\text{AUDPC} = \sum_{i=1}^{n} \frac{\left[\left(Yi + n_1 + Y_1\right)\right]}{2}\left[X_i + 1 - X_2\right]$$

where Y_i = proportion of tissue affected at the *i*th observation and X_i is time (in days) after inoculation at the *i*th observation and *n* is total number of observations. Cumulative AUDPC scores were also calculated (Jeger & Viljanen-Rollinson, 2001).

2.6 | Statistical analysis

The disease severity percentages from the different plants within a single planting date were pooled for one-way fixed effects analysis of variance (ANOVA) to evaluate the difference in disease severity between planting dates for both study plants and positive controls. ANOVAs were completed using IBM SPSS Statistics v. 28.0.1.0 (142) 20 at significance level 5%. Tukey's honestly significant difference (HSD) test was used to evaluate the differences in disease severity between planting dates at a significance level of 5%. The model fixed and random effects were unselected as ANOVA assumes that effects are fixed in independent variables. The robust tests of equality of means were done using Welch and Brown-Forsythe (Roth, 1983). General linear model was used to run univariate analysis, which tests for homogeneity and estimates of effect size using Levene's test of equality of error variances (Draper & Hunter, 1969). The null hypothesis for the Levene's test was that the error of variance of the dependent variable (disease severity %) is equal across groups (Tables S4 and S5). The influence of relative humidity, rainfall and maximum and minimum

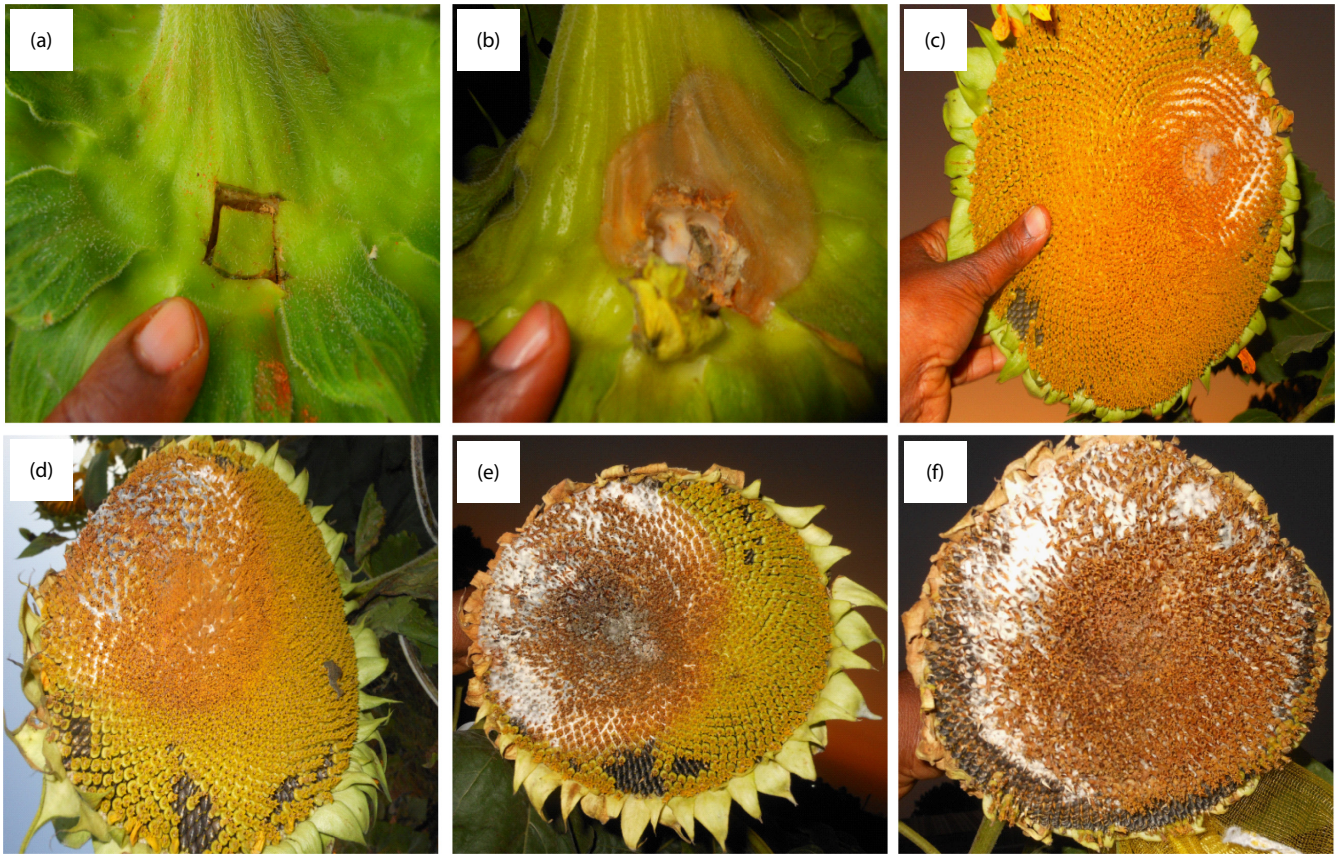

FIGURE 4 Disease severity scoring scale used to evaluate disease progression for both disease severity index and area under disease progress curve calculations. (a) 0 = no symptoms observed; (b) 1 = <12.5% of the head infested; (c) 2 = 12.5%–25%, (d) 3 = 26%–50%, (e) 4 = 51%–90% of the head infested, (f) 5 = >90% of the head infested, as described by Van Becelaere and Miller (2004). [Colour figure can be viewed at wileyonlinelibrary.com]

temperature on Sclerotinia head rot progression was evaluated with a linear progression model on XLSTAT 2023 (Lumivero) at a significance level of 5%. Negative control samples that lacked any infection were not included.

3 | RESULTS

3.1 | *S. sclerotiorum* field identification and confirmation

To ensure no additional *S. sclerotiorum* strains were introduced to our shared experimental farm, plants with symptoms characteristic of *S. sclerotiorum* infection were collected from the site in the 2020/2021 season. Sclerotia were recovered, cultured and identified with molecular markers. The ITS, *lac* and *aspr* markers confirmed that the isolates from the field station were *S. sclerotiorum*. The *lac* marker is specific to *S. minor* and was used to ensure only *S. sclerotiorum* was collected in the field and not the closely related *S. minor* species, which has also been reported in RSA (Melzer et al., 1997). We found that the *aspr* amplified the expected band in all samples, while the *lac* marker only amplified in the *S. minor* positive control, indicating all our field isolates were *S. sclerotiorum* and not *S. minor* (Figure S1).

After the field inoculation trial, we again used PCR to confirm that the pathogen from the inoculated plants was *S. sclerotiorum*, which also matched the observed symptoms. ITS PCR products were sequenced and both BLAST (100% identity) and phylogenetic analysis confirmed that all field isolates were *S. sclerotiorum* and not *S. minor* or any other related species (Figures S2 and S3).

3.2 | Inoculation technique variability

There are many different inoculation techniques available and in the literature two seemed to be viable options for our study. The mycelial spray technique more closely mimics a natural infection after ascospore germination (Bester, 2018), while the mycelial plug inoculation technique is more robust and ensures disease establishment (Chen & Wang, 2005). At the start of the trial, both methods were compared in the October and November inoculations, where the positive controls were spray inoculated and the study plants (more exposed to the elements) where plug inoculated to ensure infection. The spray-inoculated plants were covered with a paper bag to mimic an optimal environment for the pathogen and maintain higher humidity. Despite this more conducive environment, no infections were observed with this method. In contrast, the plug-inoculated

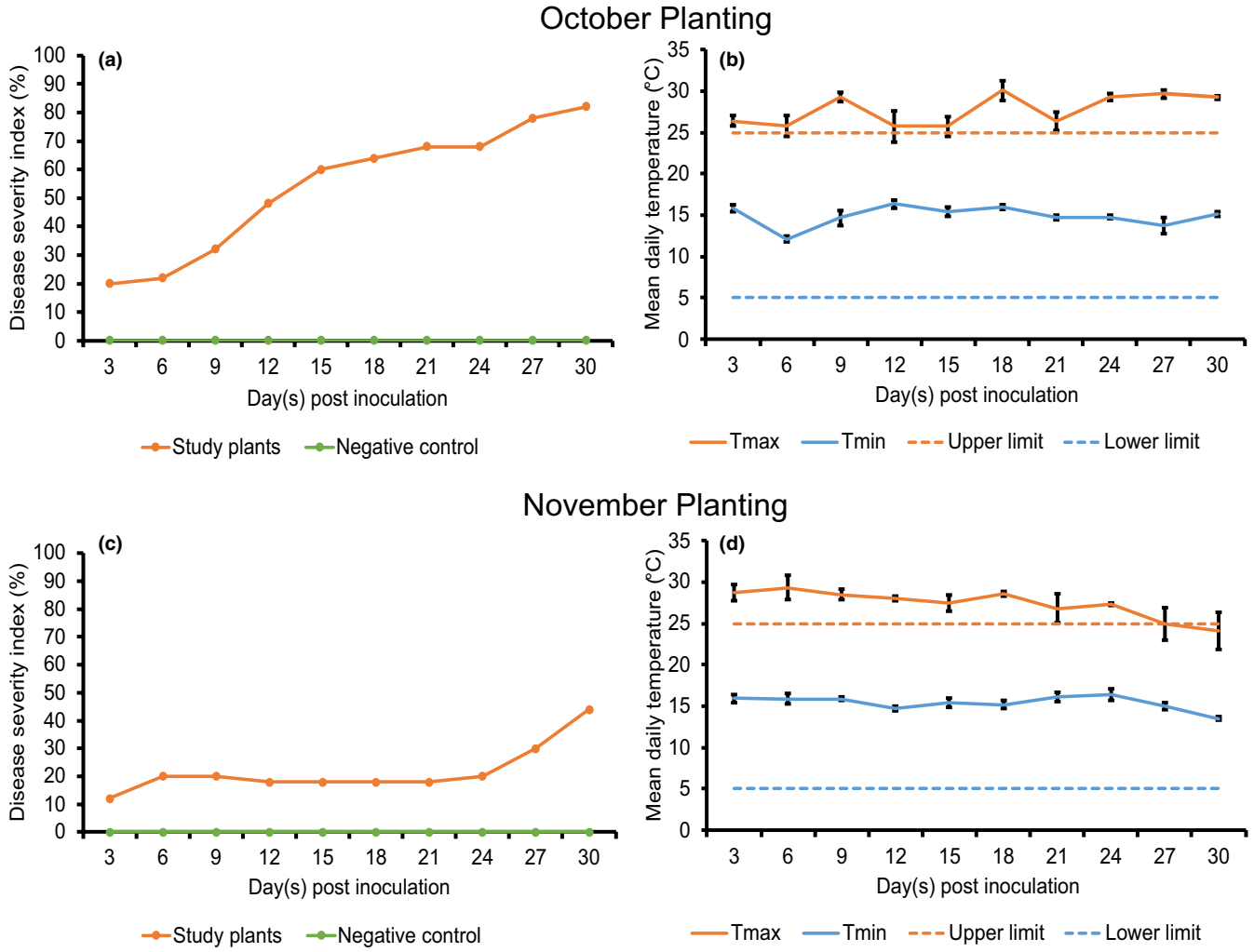

FIGURE 5 Temperatures above the *Sclerotinia sclerotiorum* threshold of 25°C suppress Sclerotinia head rot progression observed in the October (a,b) and November (c,d) planting dates. Disease severity index (%) calculated over the 30-day period after inoculation at 3-day intervals (a,c) for the test plants (orange line, $n = 10$) and negative control plants with sterile agar plug (green line; $n = 5$). Positive controls ($n = 5$) were not included due to inoculation method failure. Comparative analysis with average daytime high (T_{max}; orange line) and night-time low (T_{min}; blue line) temperatures binned across 3-day intervals for 30 days post-inoculation (b,d) shows the maximum temperatures during assessment period post-inoculation were higher than the maximum threshold temperature (dashed orange line) optimal for *S. sclerotiorum* growth, while the minimum temperatures remained within the threshold (dashed blue line) for the pathogen. Error bars represent the standard error for temperature across the 3-day bins (b,d). [Colour figure can be viewed at wileyonlinelibrary.com]

plants (particularly the October set) showed disease establishment and progression (Figure 5). This indicated that the mycelial spray inoculation method was not ideal for our experimental set up. All further inoculations were done by plug inoculation because robust disease establishment was required so that progression within the head could be monitored and compared to environmental variables across all the planting dates.

3.3 | Environmental factors limiting the establishment of Sclerotinia head rot

Initially, the study aimed to evaluate the association of Sclerotinia head rot with planting date. This trial formed part of a larger trial, which included different planting dates at two different sites across two different seasons. In both seasons at both sites, planting dates were monitored for any observations of Sclerotinia head rot (Table S1). We observed that in season 1 (2020/2021) at both sites Sclerotinia head rot occurred only in the November planting date. The second season was significantly wetter and cooler (Figure S4) and showed more variability, with the Potchefstroom trial showing signs of Sclerotinia head rot in the January and February planting dates, while at the Pretoria site, Sclerotinia head rot only occurred in the December planting date. This suggests that there is no direct link between Sclerotinia head rot occurrence and planting date across seasons or locations and it is more likely that specific microclimates in the field at the time of infection are driving establishment and progression of Sclerotinia head rot. An inoculation trial was established in the second season at the Pretoria site to begin identifying the specific local climatic factors contributing to disease establishment and progression.

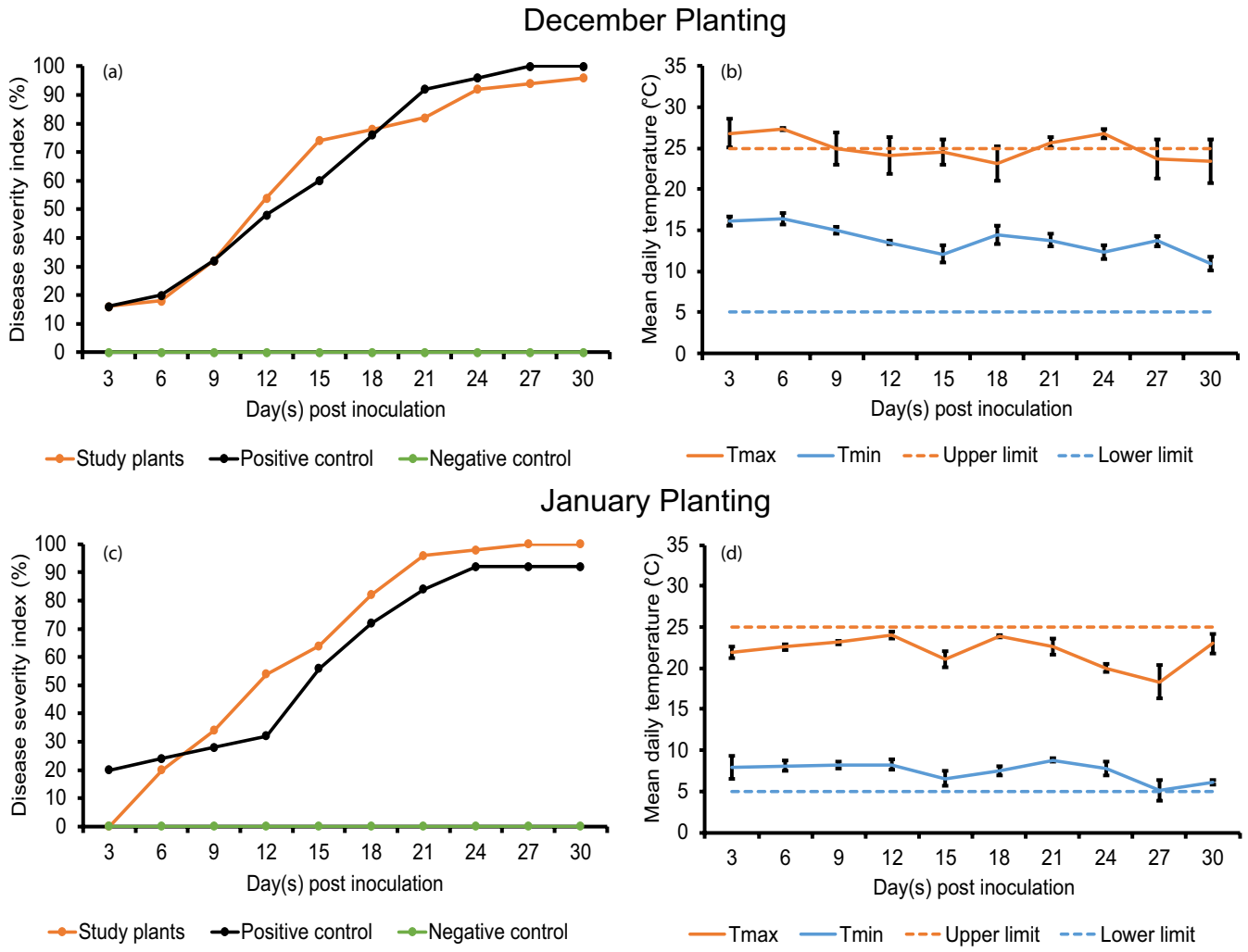

FIGURE 6 Optimal temperature conditions for *Sclerotinia sclerotiorum* growth and head rot progression observed at the December (a,b) and January (c,d) planting dates. Disease severity index (%) calculated over the 30-day period post-inoculation at 3-day intervals (a,c) for the test plants (orange line, $n = 10$), positive control plants with elevated humidity (black line; $n = 5$) and negative control plants with sterile agar plug (green line; $n = 5$). Comparative analysis with average daytime high (T_{max}; orange line) and night-time low (T_{min}; blue line) temperatures binned across 3-day intervals for 30 days post-inoculation (b,d) shows these temperatures are well within the maximum (dashed orange line) and minimum (dashed blue line) growth threshold temperatures. Error bars represent the standard error for temperature across the 3-day bins (b,d). [Colour figure can be viewed at wileyonlinelibrary.com]

Disease establishment was viewed as the time taken from inoculation to first visual symptoms on the heads, which typically took between 1 and 6 days (Figures 5–7). In most cases, for the study and positive control plants, 10%–20% DSI was observed by 3 dpi. Only three cases (January study plants, March study and March positive control plants) showed delayed disease establishment with around 20% DSI recorded at 6 dpi (Figures 6c and 7). For most planting dates, the inoculation period was characterized by optimal temperatures (between 5 and 25°C) and relative humidity (between 95% and 100%) for disease establishment (Figures 5–7 and S5).

The temperature conditions during the January inoculation set (Figure 6d) were within the optimal range for *S. sclerotiorum* (Abawi & Grogan, 1975; Uloth et al., 2015). However, we observed that at this planting date the positive control, which was covered by a paper bag to increase humidity around the head, had a DSI of 20% at 3 dpi (similar to optimal conditions). This suggests that relative humidity suppressed disease establishment in the January test plants, which is supported by the low rainfall and relative humidity recorded at the time of inoculation (Figure S5g,h). In the March planting date, both the study plants and positive controls had delayed disease establishment even though there was some rain and optimal relative humidity above 90% (Figure S5k,l). However, March is a very late autumn planting and at sunflower maturity, temperatures were dropping significantly. At the time of inoculation, the night-time temperatures were dropping well below the 5°C threshold required by *S. sclerotiorum* and could be slowing disease establishment for this planting date (Figure 7c,d).

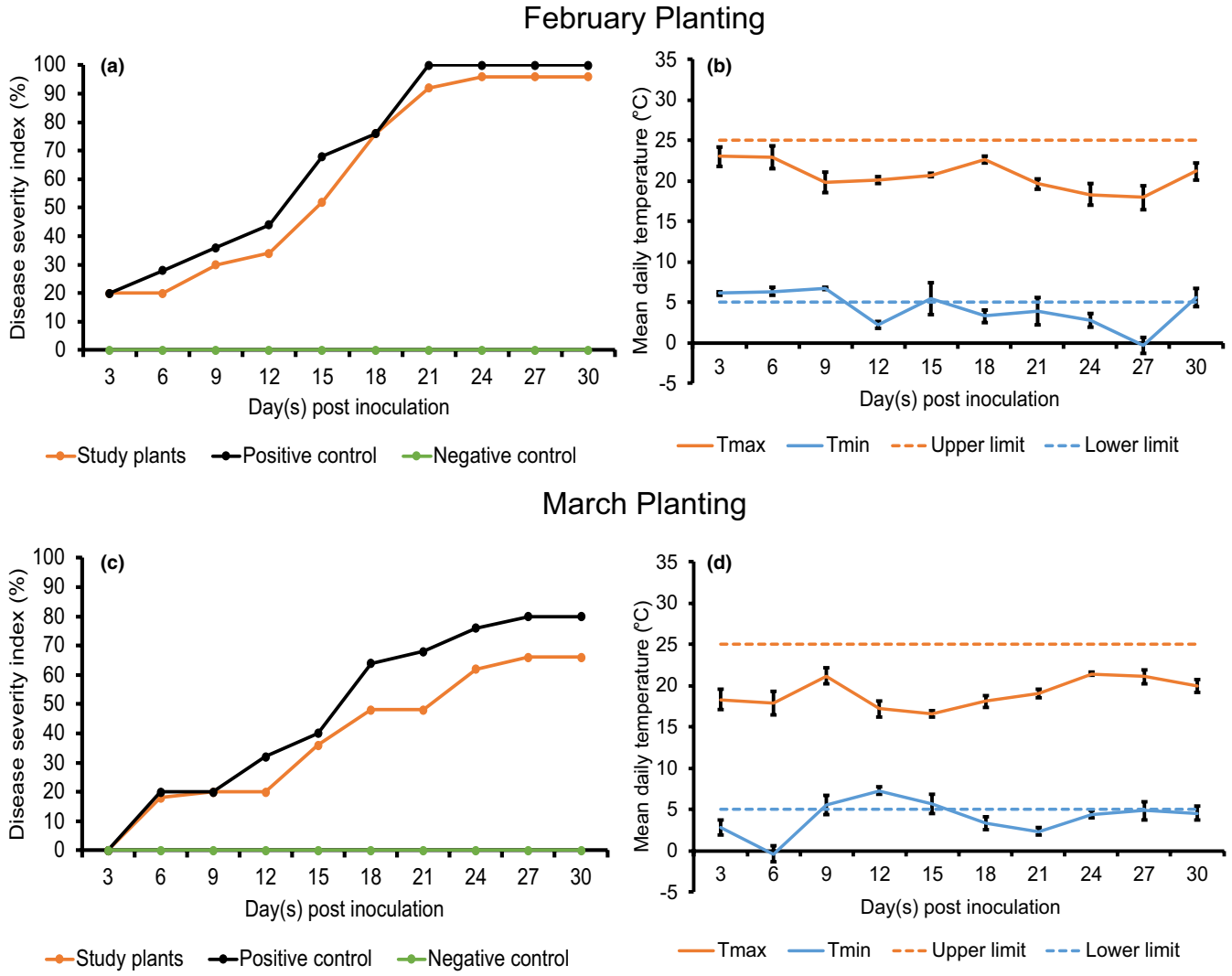

FIGURE 7 Temperatures below the *Sclerotinia sclerotiorum* minimum threshold of 5°C suppress Sclerotinia head rot progression as observed in the February (a,b) and March (c,d) planting dates. Disease severity index (%) calculated over the 30-day period post-inoculation at 3-day intervals (a,c) for the test plants (orange line, $n = 10$), positive control plants with elevated humidity (black line; $n = 5$) and negative control plants with sterile agar plug (green line; $n = 5$). Comparative analysis with average daytime high (T_{max}; orange line) and night-time low (T_{min}; blue line) temperatures binned across 3-day intervals for 30 days post-inoculation (b,d) shows that minimum temperatures below the growth threshold (blue dashed line) for *S. sclerotiorum* suppress sclerotinia head rot progression even if the maximum temperatures are within the maximum threshold. Error bars represent the standard error for temperature across the 3-day bins (b,d). [Colour figure can be viewed at wileyonlinelibrary.com]

3.4 | Effect of temperature on Sclerotinia head rot progression

Our results can be grouped by temperature changes across the season, where the early spring/summer planting dates (October and November) were characterized by higher temperatures and lower relative humidity (Figures 5 and S5), the midsummer planting dates (December and January) were characterized by moderate-high temperatures and high relative humidity (Figures 6 and S5), and the late summer/autumn planting dates (January and February) were characterized by significantly lower temperatures and low rainfall (Figures 7 and S5). This variation across the season had different effects on the disease progression, with temperature appearing to be a strong driver of disease progression.

The conditions during the inoculation trial for the December and January planting dates were optimal for *S. sclerotiorum* growth, with temperatures around or within the 5–25°C thresholds for the pathogen and relative humidity between 90% and 100% (Figures 6b,d and S5e–h). The study plants and the positive controls all reached between 90% and 100% DSI between 24 and 27 dpi (Figure 6a,c). This suggests, as other studies have (Clarkson et al., 2014), that these conditions are optimal for Sclerotinia head rot progression.

The October and November planting date inoculation and monitoring periods were significantly hotter than the other planting dates

assessed and were both consistently above the 25°C maximum threshold reported for *S. sclerotiorum* (Figure 5b,d). The October planting date showed a lower final DSI of between 80% and 90% at 30 dpi compared to the January and December planting dates, which reached over 90% DSI 3–6 days earlier (Figure 6a,b). The November planting date, which was characterized by much hotter days, had the lowest DSI across all planting dates and only reached a maximum of 44% at 30 dpi (Figure 5b). In some cases, inoculated plants even recovered and showed no disease symptoms, suggesting high temperatures can suppress and even stop Sclerotinia head rot progression.

Extreme cold temperatures below the minimum 5°C threshold for *S. sclerotiorum* do appear to have an impact on Sclerotinia head rot establishment and progression; however, the effect is more nuanced than for high temperatures. The inoculation and monitoring period for the February planting date had minimum daily temperatures more consistently below the 5°C threshold compared to the March planting date (Figure 7b,d); however the February planting date had good disease progression and both the study and positive control plants reached 95%–100% DSI by 24 dpi comparable with the progression observed for the December and January planting dates (Figures 6a,c and 7a). In contrast, the March planting date had a severe cold snap at the time of inoculation, which suppressed disease establishment and even though temperatures recovered, the disease progression remained slow, only reaching 80% DSI for the positive controls and 66% DSI for the study plants at 30 dpi (Figure 7c,d). Relative humidity does not explain these results as the February planting date inoculation period appeared to have more days with lower relative humidity compared to the equivalent period for the March planting date (Figure S5). The suppressed disease progression is likely a combination of the severe cold snap delaying disease establishment at the time of the March planting date inoculations and the consistently lower day time temperatures over the disease monitoring period. The T_{max} temperatures for the February inoculation trial fluctuated generally between 20 and 25°C, while for the disease monitoring period of the March planting date it fluctuated between approximately 15 and 20°C (Figure 7).

The cumulative AUDPC trends and the final AUDPC scores support the trends observed by DSI, where the disease progression for December, January and February produced AUDPC values between 84 and 90. The warmer October and November periods produced lower AUDPC values of 75.15 and 29.4, respectively. The cold autumn March planting date also produced a lower AUDPC score of 52.6 (Table S6). Taken together, these results show that both high and low temperatures can be important suppressors of Sclerotinia head rot progression.

Multiple linear regression analyses supported the disease progression results (Figures 5a, 6a,c, and 7a,c,e) indicating that rainfall, daytime relative humidity (H_{max}) and maximum/minimum temperatures most influenced Sclerotinia head rot progression. According to the type III sum of squares, average rainfall was the most influential weather variable on Sclerotinia head rot progression for the October planting date (Table S6), while Sclerotinia head rot progression in the November planting was highly influenced by mean daily maximum temperature (Table S6). In the December, February and March planting dates, mean daily minimum temperature (Figure 7b,d,f) had the strongest influence on Sclerotinia head rot progression (Table S6). Daytime relative humidity (Figure S5g) had a positive influence on the progression of Sclerotinia head rot at the January planting date (Table S6).

An analysis of covariance revealed that the planting date strongly affected Sclerotinia head rot progression (at a significant level of 95%). According to the type III sum of squares, date of planting was the most influential variable on Sclerotinia head rot progression ($p = 0.032$, $R^2 = 0.291$, Table S7). Together, the AUDPC, linear regression and analysis of covariance indicate that the planting date has a significant effect on Sclerotinia head rot progression, but the individual climatic drivers of head rot progression differ from planting date to planting date (Figures 5 and 6, Tables S6 and S7).

3.5 | Effect of temperature on Sclerotinia head rot severity

Disease severity is the volume or area of visibly diseased plant tissue relative to the total plant tissue (Campbell & Madden, 1990). Sclerotinia head rot was most severe in the December, January and February planting dates (Figure 8a), but less severe in inoculated plants of the November planting (Figure 8a), with the maximum temperature (Figure 5b) suppressing disease progression and consequently the disease severity. ANOVA results showed highly significant differences ($p < 0.0001$) in Sclerotinia head rot severity across the six planting dates, with mean DSI score ranging between 2.2 for the November planting date to 5 for the January planting date (Figure 8a). January versus November, February versus November and December versus November planting dates showed highly significant differences ($p < 0.0001$), indicating that November was unique in terms of the lower DSI scores (Figure 8a). The positive controls were severely affected by Sclerotinia head rot as shown by the analysis of variance results (Figure 8b) at a significance level of 95%, as expected.

The Levene's test results indicated unequal variances, with $F = 37.479$ and corresponding $p < 0.007$ for the study plants, and $F = 96.00$ and corresponding $p < 0.001$ for the positive control, leading to rejection of the null hypothesis, which stated that the error variance of the dependent variable (disease severity percentage) is equal across groups. There is sufficient evidence that the variance in disease severity between planting dates is significantly different (Table S4). Planting date had a large effect on disease severity in both study plants and positive controls, with partial eta squared (η^2) value of 0.563 (56.3%) and 0.529 (52.9%), respectively (Table S5). The study and positive control plants for the March planting date showed a significant difference in disease severity. These findings support the linear regression analysis (Table S7), indicating that planting date significantly affects the establishment, progression and severity of Sclerotinia head rot.

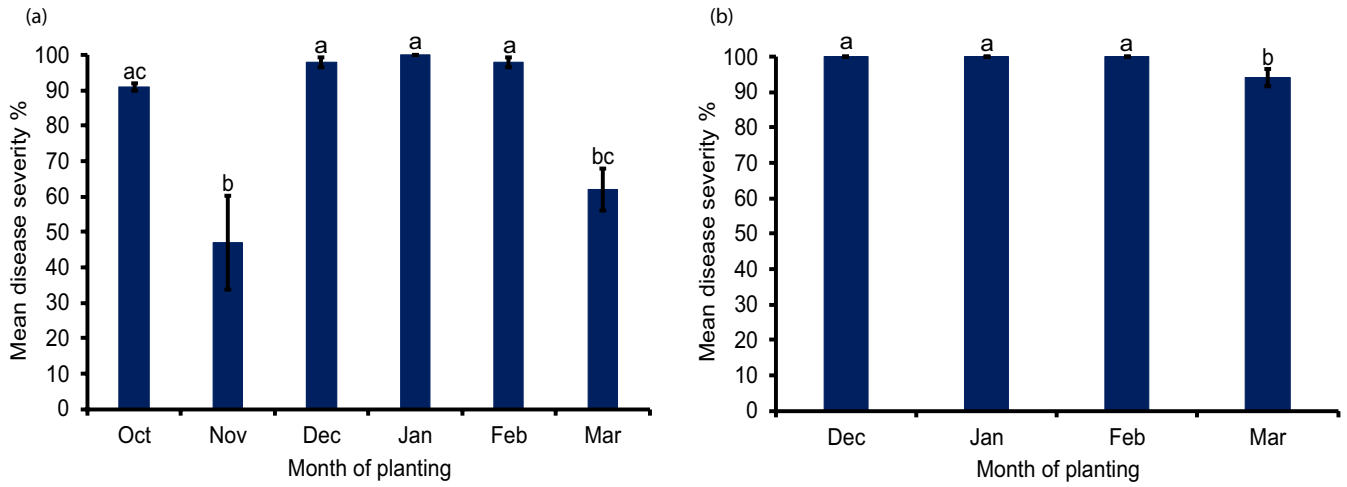

FIGURE 8 One-way analysis of variance (ANOVA) results revealed that there were significant differences in percentage disease severity between the test plants across planting dates at $p < 0.001$ (a), and a significant difference in disease severity percentage only for the March planting date of the positive control plants at $p < 0.05$ (b). Significance determined by Tukey's honestly significant difference (HSD) post hoc test and Levene's test of equality of error variances (Tables S4 and S5). The same letter above the error bars indicates nonsignificant difference between planting dates; the error bars represent standard error, for test plants $n = 10$ (a) and for controls $n = 5$ (b). The disease severity percentage used for one-way ANOVA was collected at 33 days post-inoculation across planting dates. [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

Sunflower production in RSA is impacted negatively by diseases, such as Sclerotinia head rot. Short-interval, staggered sunflower plantings have been suggested as an escape strategy to limit the number of plants at flowering stage when environmental conditions are conducive for Sclerotinia head rot (Bureau for Food and Agricultural Policy, 2020). However, these planting strategies bring several challenges, including complicating field management practices. With dryland agricultural practices, as is common in RSA (Meyer & van der Burgh, 2015), short-interval, staggard plantings can be difficult when late rains push plantings towards the end of the planting window. In this study, we have investigated the influence of climatic factors and planting dates on Sclerotinia head rot progression across the entire summer season to evaluate how planting date might impact Sclerotinia head rot establishment and progression.

In a broader planting date trial, we observed *Sclerotinia* disease occurrences to vary across location or season and these were not consistently associated with a specific planting date, probably due to the interseasonal weather variability in RSA. RSA has a high interseasonal variability often brought about by the wet and dry cycles as the region transitions between El Niño and La Niña events (Ndlovu et al., 2021; Rusere et al., 2023). *S. sclerotium* has specific requirements in terms of temperature and relative humidity at specific stages of its life cycle (Bečka et al., 2016; Clarkson et al., 2014; Harikrishnan & Rio, 2008; Payen, 1983; Smolińska & Kowalska, 2018), and the changes can be small and only required for a short time to induce disease establishment. These factors make seasonal generalizations difficult and probably contribute to the erratic nature of Sclerotinia head rot outbreaks, particularly in RSA (Crave et al., 2016; Holtzhausen & Van der Westhuizen, 1980; Meiring et al., 2021). We therefore implemented a small inoculation trial in a single season and location to better understand the in-field local climatic factors that drive disease establishment and head rot progression within the sunflower head at different planting dates. While the temporal occurrences of these optimal conditions for *S. sclerotiorum* growth might shift from season to season and location to location, their influence on disease progression should remain similar and may provide insights into which factors are key to infection.

We systematically inoculated randomly selected plants in monthly plantings across the summer season and monitored disease progression within the sunflower heads at 3-day intervals so that we could directly compare progression of disease to the prevailing climatic conditions at each time point. In this trial, we observed a strong correlation between planting date and Sclerotinia head rot progression and we showed that different climatic factors drive this association at different planting dates. Generally, we found that disease establishment after inoculation was suppressed by low relative humidity and extreme cold conditions occurring at the time of inoculation. While disease progression within the heads was significantly suppressed by high temperatures, cold conditions could suppress Sclerotinia head rot only if both the night- and daytime temperatures were low.

In this study, we considered disease establishment as the 6-day period after inoculation where lesions first start to be observed on the flower head. In a natural *S. sclerotiorum* head rot infection, an ascospore will land on the flower head, germinate, enter the head via senescing florets and the mycelial growth will advance across the entire head (Bečka et al., 2016; Harikrishnan & Rio, 2008; Payen, 1983). Here, we placed mycelia directly into the back of the flower head so that we could assess how the mycelia

establish and progress across the head and identify the climatic factors contributing to these processes. We noted two interesting deviations from good disease establishment in our trials. The first was in the January planting date, where the study plants that were inoculated and exposed to the environment had slow disease establishment and lesions were only observed after 6 dpi. However, the positive control plants that were inoculated and covered with a paper bag to maintain humidity around the flower head showed lesions forming more rapidly (before 3 dpi). Similarly, for the very late March planting date we noted that both the positive control and study plants had suppressed lesion development, with the first lesions developing between 3 and 6 dpi. This suggested that local relative humidity was not an influencing factor, but rather the severe cold snap (approximately 0°C night-time temperatures at the time of inoculation) suppressing the disease establishment in both sets of plants. While our inoculation strategy does not allow for the study of ascospore attachment or germination and entry to the head, these processes have been found to occur at relative humidity of more than 85% and between 5 and 25°C (Clarkson et al., 2014). It is reasonable to assume that the lower humidity in our January planting date and extreme low temperatures in the March planting date could affect these processes in a natural infection as well.

The next phase investigated head rot progression, which was measured from inoculation to 30 dpi, when most heads were completely infected. In terms of disease progression, the data can be separated based on progression and temperature, which related to time of the season for each planting date. The spring and early summer planting dates (October and November) showed the slowest disease progression, only reaching AUDPC scores of 75.15 and 29.4, respectively. When we compared this to the daily temperature maxima and minima during the monitoring period, we observed that in both months the maximum temperatures during the inoculation and monitoring period were consistently above 25°C. The natural growth range for *S. sclerotiorum* mycelial growth is between 5 and 25°C, and temperatures of 27°C or higher have been shown to be detrimental to disease progression (Abawi & Grogan, 1975; Ebrahimi et al., 2013; Smolińska & Kowalska, 2018; Weiss et al., 1980). In the November planting date inoculation, some plants showed no symptoms or presented with recovery after inoculation, resulting in the very low DSI and AUDPC scores. No other planting dates showed inoculation failure or plant recovery for any of the plants inoculated. It is likely that for this planting date, the high temperatures placed the fungal pathogen under significant stress, while these temperatures were more favourable for the sunflower plants providing an opportunity to counter the infection and recover (Velásquez et al., 2018). Rondanini et al. (2006) reported that average temperatures consistently over 30°C begin to influence yield and oil production in sunflower. This suggests that selecting a sunflower planting date that ensures flowering aligns with the hottest parts of the summer season may help limit *Sclerotinia* disease progression in regions where the summers average over 27°C consistently without major impacts on production.

The December, January and February planting dates presented with the fastest disease progression, with AUDPC scores ranging between 84 and 90. The DSI scores also showed fast progression, with scores between 90% and 100% by 24 dpi. These results are expected if we look at the climatic variables associated with the inoculation period of these planting dates. Where the December and January planting dates showed relative humidity between 90% and 100%, February had a slightly wider range from 85% to 100%. This is around the optimal relative humidity for *S. sclerotiorum* growth and development (Clarkson et al., 2014). Similarly, the optimal growth range for *S. sclerotiorum* is between 5 and 25°C. The average maximum temperatures for the inoculation period of the December planting date fluctuated around an average of 25°C, while the January and February periods fluctuated around 20 to 25°C, well within the optimal temperature range (Clarkson et al., 2014). This may present with some concern as the standard RSA production guide proposes sunflowers are normally planted between November and January (Anonymous, 2009). Even when we look at the results of the greater planting date trial across two seasons and two sites where the 2020/2021 season was hotter and drier than the second, we observed Sclerotinia head rot within this planting window, with a possible early shift in the hotter drier season. It may be useful to monitor seasonal forecasts and try to identify the hottest months in different seasons, to try and limit Sclerotinia head rot by aligning planting dates within this window.

The late summer and early autumn planting dates of February and March showed that low daily maximum and minimum temperatures together suppress Sclerotinia head rot progression, while low daily minimum temperatures alone are not sufficient for suppression. While interesting in terms of the biology of the pathogen, which has been well studied in the past (Imolehin et al., 1980; Uloth et al., 2015), this does not have much in the way of agricultural implications, given freezing conditions may limit growth and development in even the hardiest frost-tolerant crop plant (Mangin et al., 2017). Sunflower has consistently been shown to be more susceptible to cold snaps and freezing temperatures, which significantly limit yield (Hammer et al., 1982; Hniličková et al., 2017). There are several breeding efforts to improve frost tolerance in sunflower, possibly by using introgression from wild species (Tetreault et al., 2016) and perhaps this will alter the planting window and provide flexibility to aligned plantings and flowering stages with environments less conducive to Sclerotinia head rot in the future.

In conclusion, we found that planting date was correlated with Sclerotinia head rot progression, but that this varied across seasons and locations and was influenced by different climatic variables including relative humidity and temperature. We showed that disease establishment by mycelia in the sunflower head is suppressed by low humidity and severe cold, while Sclerotinia head rot progression was suppressed by extreme high or low temperatures. To limit adding additional *S. sclerotiorum* strains or inoculum to our shared field site, we used a single isolate that we collected from the same site the previous

year and used molecular tools to confirm the identity and produce a pure culture. It would be interesting in future studies to test other isolates with varying levels of pathogenicity. We also used the plug inoculation technique because it was robust and allowed us to investigate head rot progression across all planting dates. Several members of our team are focused on developing a reliable protocol for ascospore production, and it will be interesting to repeat this trial using a more natural mode of infection in the future. It would also be interesting to repeat this trial in the coming seasons as RSA is transitioning back into a hotter drier period with El Niño returning (Sihlobo, 2023).

Bester (2018) evaluated several inoculation techniques and focused on understanding the effect of short-interval, staggered plantings on Sclerotinia head rot incidence in RSA, while our study focused specifically on understanding how environmental factors impact Sclerotinia head rot progression across monthly planting dates to assess the entire summer season. This is the first study of its kind and presents important findings on which weather variables should be considered at flowering to limit Sclerotinia head rot progression. We show that high temperatures suppress disease progression and aligning plantings to ensure bloom periods coincide with the hottest part of the season may aid in suppression of Sclerotinia head rot. This study provides a solid base from which to further assess strains, infection processes and other environmental conditions, which is critical knowledge for the development of more effective control strategies.

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DATA AVAILABILITY STATEMENT

The data are available upon request from the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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