Prevalence of *Botrytis cinerea* at different phenological stages of table grapes grown in the northern region of South Africa

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Highlights

- *Botrytis cinerea* is monitored at different phenological stages of table grapes at different agro-climatic sites.
- *Botrytis cinerea* is detected in all evaluated phenological stages using ddPCR on asymptomatic samples.
- Full boom stage of table grapes showed the most prevalence of *Botrytis cinerea* in high rainfall site.
- Early preharvest monitoring of *B. cinerea* can assist growers reduce postharvest decay.

ABSTRACT

Botrytis cinerea, is one of the major causal agents of postharvest decay in table grapes, contributing to 20% losses world-wide. The aim of this study was to determine the prevalence of *B. cinerea* at different phenological growth stage (full bloom, pea size and fully mature berries)

and the impact of agro-climatic sites on pathogen incidence. Droplet digital polymerase chain reaction (ddPCR) was used simultaneously with culture based methods to detect and quantify *B. cinerea*. *Botrytis cinerea* was detected at different phenological stages on asymptomatic grape samples. Prevalence and level of *B. cinerea* differed between phenological stages and sites. Full bloom stage of site B showed the highest prevalence of *B. cinerea* (82.9%) compared to site A (33.3%). For the latter phenological stages (pea size and mature stage), site A had the highest prevalence (100% for both), compared to 35.2% and 44.4% observed at site B, respectively. Furthermore, the *B. cinerea* concentration varied between stages within the two sites. The concentration of *B. cinerea* at site A showed a threefold increase from pea size (2.67 copies μL^{-1} of DNA) to mature berry stage (9.16 copies μL^{-1} of DNA), compared with the decline noted for similar growth stages at site B. *Botrytis cinerea* inoculum build up on asymptomatic grapes could be effectively monitored as the berry develops at critical phenological stages. Preharvest monitoring of the pathogen can help growers improve well-established cultural and management practices, hence limit the risk of postharvest decay.

Keywords: full bloom, mature berries, microbial ecology, pea size, postharvest pathogens, preharvest.

1. Introduction

Botrytis cinerea, Pers.: Fr, a fungal pathogen that causes gray mold, is regarded as one of the most economically important postharvest pathogens of table grapes (Gubler et al., 2013), blueberries (Rivera et al., 2013), strawberries (Feliziani and Romanazzi, 2016) and other fresh

produce (Carisse and Van Der Heyden H, 2015). The pathogen, *B. cinerea*, was placed second in a list of the world's top fungal plant pathogens, due to its importance scientifically and economically (Dean et al., 2012). McClellan and Hewitt (1973) described the different infection pathways of *Botrytis*, as one of the infections occurring at full bloom and, through the floral parts (petals, styles, stigmas or stamen). Established fungal hyphae of *B. cinerea* remains dormant during pre-harvest phenological stages until conducive environmental conditions such as fruit injuries and high sugar content enable pathogen proliferation (Romanazzi et al., 2016). Infections of *B. cinerea* that are not detected at harvest, during packing and/or transportation may lead to the growth and subsequent spread of the disease in table grapes postharvestly even when stored at low temperature (-0.5°C), thereby, reducing the market value of the product (Crisosto et al., 2002; Celik et al., 2009). Abundant sporulation may arise from a single infected berry and contaminate the entire batch of grapes (Romanazzi et al., 2016) leading to the manifestation of the fungus later in the supply chain. As a result, the pathogen is responsible for severe economic damage accounting for 20 % postharvest loses world-wide, valued between 10 and 100 billion Euros per year (Anonymous, 2015).

To reduce such losses, several technologies are available for the control of gray mold including the use of fungicides (Feliziani et al., 2014) and sulfur dioxide (Gándara-Ledezma et al., 2015). Despite pressure to develop alternative applications, some registered active ingredients are still used to control gray mold on different crops, including table grapes. However, several conventional fungicides have been banned across the world especially in Europe (Romanazzi et al., 2016), a market accessed by the South African table grape industry. In this context early, rapid, and accurate detection of *B. cinerea* in table grapes is essential for, amongst others, developing disease prediction models and alternative control applications.

Among the many culture independent approaches recently used (Saito et al., 2013; Jongman et al., 2017), a highly sensitive detection tool, the droplet digital Polymerase Chain Reaction (ddPCR) which does not require calibration standards (Flatschart et al., 2015; Koppel and Bucher, 2015; Pinheiro et al., 2012) has since been introduced. This recent technology was commercialised to allow precise quantification and detection of the target nucleic acid in a sample. It determines absolute concentrations of nucleic acid copies in discrete, volumetrically defined, water-in-oil droplet partitions of a sample into 20000 smaller segregated reactions. Then using a standard PCR reaction method, the target is amplified in each partition and individually counted as positive or negative by the associated target dependent florescence signal. It further offers a simple workflow capable of generating highly stable partitioning of DNA molecules (Pinheiro et al., 2012).

Most studies using ddPCR have mainly focused on clinical research areas such as cancer (Albano et al., 2015; Beltrame et al., 2015; Combaret et al., 2015) and human saliva (Bahn et al., 2015). Other applications of the ddPCR include quantification of potato bacterial pathogens (Dreo et al., 2014), *Phytophthora nicotianae* (Timbo et al., 2004), bovine viral diarrhea viruses (Flatschart et al., 2015), and analysis of genetically modified organisms (Koppel and Bucher, 2015). However, to our knowledge its application in plant pathology is still new and more specifically in table grape or other fruit types in preharvest environments is limited.

In our study, the ddPCR, was used to detect and quantify *B. cinerea* in asymptomatic table grape tissues. The aim of the study was to determine the prevalence of *B. cinerea* using the ddPCR absolute quantification method at different berry developmental stages. In addition, the effect of different agro-climatic sites on prevalence and incidence of the pathogen was determined.

2. Materials and Methods

2.1 Site description and sample collection

Samples were collected from vineyards located in the northern table grape production region of South Africa during the 2015/16 season. Two commercial farms (site A and B) were selected for this study. These two sites differ with respect to geographic location and climatic conditions, as described in Carmichael et al (2017). Site A is at a lower altitude (899 m above sea level) than site B (1123 m above sea level). Annually, site B receives higher average rainfall (350 – 700 mm) than site A (150 – 350 mm). The cultivar used was 'Crimson Seedless' grafted on R110 rootstock. Other crops cultivated at both sites included citrus and water melon.

A split plot design was applied, with six replicates per site and three subplots per replicate. Three experimental vines were randomly selected in each subplot. A total of 263 samples comprising of full bloom (stage 23) (n = 47), pea size (stage 31) (n = 108) and mature stages (stage 38) (n = 108) according to the modified E-L scale (Combe, 1995) were collected. Sampling was done at three points around the vine, representing East, West and inside canopy to harmonise the effect of micro-climate per sample. Following the same strategy, bioaerosol samples were collected by direct impaction using an air sampler (SAS Super 100, Cherwell laboratories, England). All samples were collected aseptically with disposable sterile gloves, placed in sterile brown paper bags and were immediately transported to the laboratory in a cooler box with ice packs. Analyses were initiated within 24 h. Weather data to define the differences for the two sites at the time of sampling was obtained from the South African Weather Services.

2.2. Measurement of physiological maturity parameters at harvest

Berry mass (g), firmness (N), and total soluble solids (TSS) (%) were measured on five individually selected berries of each sample at commercial harvest stage. Berries were carefully detached by cutting the pedicels. For berry mass, individual berries were weighed on a calibrated scale (Mettler instrumente AG CH-8606 Greifensee - Zürich, Switzerland). Firmness was determined using a penetrometer. The berry diameter was determined using a tape measure along the transversal section of each berry. Total soluble solids (%) were measured from extracted juice using a hand digital refractometer (PR-32, Atago, TSS 0-32 %, Palette, Tokyo, Japan).

2.3. Isolation of Botrytis cinerea from table grape flowers and berries

Methods described by Diguta et al. (2010) were followed with modifications. Table grape samples (flowers and berries), 25 g, were placed in a beaker containing 225 mL sterile peptone buffered water (PBW) (Merck, Johannesburg, South Africa) supplemented with 0.025 % (v/v) Tween 80 (Associated Chemical Enterprises, Johannesburg). This was then partially submerged in an ultrasonic water bath (Labotec, Johannesburg) sonicated for 5 min. at 25 ^{o}C to dislodge microbes. A 1 ml of the washing was used in a standard dilution series in PBW up to 10^{4}. Subsequently 0.1 ml washing was plated on 90 mm plates with Malt Extract Agar (Merck, Johannesburg) (detection limit, 1 $CFUg^{-1}$). All plates were incubated at 25 ^{o}C for four days, after which fungal colonies were counted and grouped according to visual appearance and representatives of each group were isolated and purified. All isolated fungi were maintained in sterile water kept at room temperature. The remaining microbiota washing (224 ml) was concentrated by filtration through a 0.45 µm pore size nitro-cellulose membrane (Sartorius Stedim Biotec, Goettingen, Germany). The filter was then stored at 4 ^{o}C before DNA extraction.

2.4. DNA extraction

The DNA was extracted directly from the filter paper using the fungal / bacterial Zymo Research kit (ZymoReseach, USA), as per manufacture's specifications. The eluted DNA was quantified using Qubit® Fluorometer (dsDNA HS (High Sensitivity Assay Kit)) (Lifescience Technology, Johannesburg). Isolated DNA was stored at -20 ^{o}C for further experimental procedures.

2.5. Controls and method optimization

To test the sensitivity of the primers, DNA from *Alternaria alternata* (Fr.) Keissl. (PPRI 10993, Biosystematics, Agricultural Research Council, South Africa) isolated from *Solanum lycopersicum* was used as a negative template control. Template DNA of *B. cinerea* isolated from 'Thompson Seedless' table grapes was used as a positive control and identified by the diagnostic centre of the Forestry and Agricultural Biotechnology Institute, University of Pretoria. In order to obtain a clear separation between the negative and positive droplets from the ddPCR output, optimization of the positive control to the best amplitude was necessary. Three dilutions, (1:10, 1:100 and 1:1000) of the positive control with sterile sabex water were tested. The 1:10 diluted positive template had the best amplitude (results not shown), and was used for the ddPCR analysis.

2.6. Droplet digital PCR assays

The DNA from the filter papers was used to detect and quantify *Botrytis cinerea* using the QX100^{TM} Droplet Digital^{TM} PCR system (Bio-Rad, Pleasanton, CA, USA) (detection limit, 1

DNA copy μL^{-1}). The reaction mix for ddPCR consisted of 1 µL DNA, 10 µL Supermix (2xQX200 EvaGreen Mix) (Bio-Rad), 8.6 µL sterile H_2O and 0.2 µL of each primers. Primers specific to *B. cinerea* (Bc3_F: 5′-GCTGTAATTTCAATGTGCAGAATCC-3′; Bc3_R: 5′-GGAGCAACAATTAATCGCA TTTC-3′) (GenBank: AM233400.1) (Suarez et al., 2005) were used. All samples were analysed in duplicates. After droplet generation, 40 µL of the generated droplet emulsion was loaded into a 96 well PCR plate. PCR was performed in a T100™ thermal cycler (Bio-Rad). The amplification conditions were 5 min. at 95 °C DNA polymerase activation, followed by 39 cycles of 30 s at 95 °C for denaturing and 60 s at 60 °C for annealing and extension, followed by a final hold of 5 min. at 90 °C for droplet stabilisation and cooling to 4 °C. After thermal cycling, the plate was transferred to a droplet reader (Bio-Rad), and the data was generated using QuantaSoft version 1.7.40917, provided with the ddPCR system.

2.7. Data and statistical analysis

The ddPCR data was first analysed in QuantaSoft™ software (Bio-Rad) version 1.7.40917 following manufacturer's guidelines. Wells with less than 10,000 accepted droplets were not included in the analysis. Calculation of the target concentrations in copy number per µl was automatically done by QuantaSoft. The data was then statistically analysed using General Linear Models procedure of Statistical Analysis Systems (SAS) version 9.4 (Institute Inc., Carry NC, USA) to determine differences between sites, phenological stages and vineyard blocks within a site. Pearson's correlations were also used to correlate physiological quality at harvest and concentrations of *Botrytis cinerea.* In order to further define the relationship between the prevalence rate and the physiological changes during fruit development, a linear regression analysis was applied.

3. Results

3.1. Prevalence and incidence of Botrytis cinerea during table grape development

The prevalence of *Botrytis cinerea* was determined by the percentage positive samples at each phenological stage. Differences in the prevalence of *B. cinerea* between the two agro-climatic sites were noted. Of the 263 samples analysed in this study, 70.3 % were positive for *B. cinerea*. Pea size and mature stage samples from site A had the highest prevalence (98.2 %) of *B. cinerea* compared to full bloom stage samples (33 %) from the same site. The prevalence of *B. cinerea* on table grape full bloom, pea size and mature stage samples from site B was 83 %, 35 % and 44 %, respectively (Fig. 1).

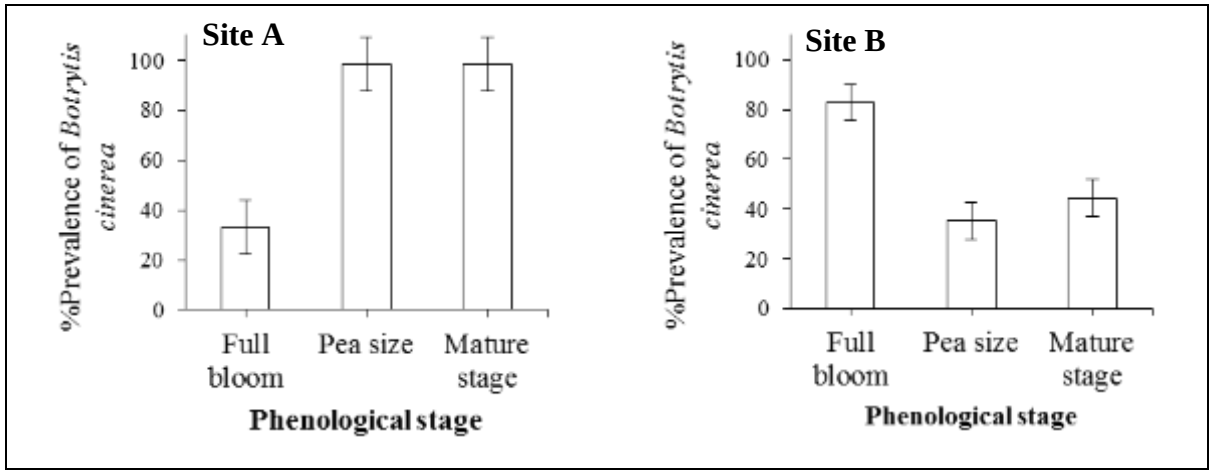

Fig. 1. Prevalence of *Botrytis cinerea* on table grape samples from two commercial farms at full bloom, pea size and mature stages. Error bars show standard error.

The average concentration of *B. cinerea* on full bloom samples from site B (15.6 copies μL^{-1} of DNA) was higher compared to those from site A (0.9 copies μL^{-1} of DNA). On average, the concentration of *B. cinerea* at pea size and mature stages was 1.8 and 4.7 copies μL^{-1} of DNA, respectively. At site A, the level of the pathogen detected on berries at pea size (2.7 copies

μL^{-1} of DNA) and mature (9.2 copies μL^{-1} of DNA) stages was higher compared to site B (Fig. 1). The concentration of *B. cinerea* in pea size and mature berry samples from site A and site B were significantly different (Fig. 1). The highest concentration of *B. cinerea* was detected on flower samples from site B (15.6 copies μL^{-1} of DNA) and mature berry samples from site A (9.16 copies μL^{-1} of DNA). The sampled blocks (Fig. 2) in the vineyard showed differences in the concentration of *B. cinerea*. Blocks locates in the central part of the vineyard were associated with higher levels of *B. cinerea* compared to those in the periphery.

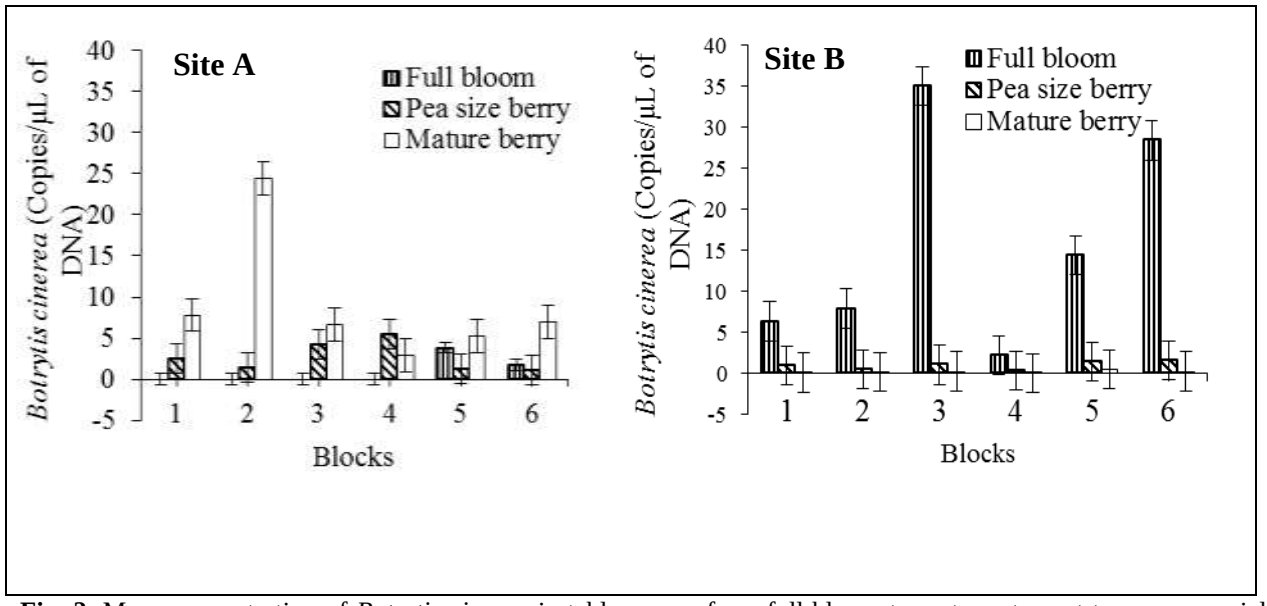

Fig. 2. Mean concentration of *Botrytis cinerea* in table grapes from full bloom to mature stage at two commercial sites. Error bars show standard error.

3.2. Table grape physiological quality for the two agro-climatic sites at maturity

The berry physiological quality at maturity differed between the two sampling areas. Berries from site A were larger in size (6.6 g per berry and 21.1 mm diameter) than those from site B (5.5 g per berry and 20.4 mm). Furthermore, larger sized berries from site A were

associated with lower firmness and TSS compared to the smaller sized berries harvested from site B (Table 1).

Table 1. Physiological parameters of table grapes at harvest[β]

	Site A[α]				Site B			
Block	**Berry mass (g)**	**BD[a] (mm)**	**Firmness (N)**	**TSS[b] (%)**	**Berry mass (g)**	**BD[a] (mm)**	**Firmness (N)**	**TSS[b] (%)**
1	6.36 ±0.94	20.68 ±0.75	5.87 ±0.69	17.28±1.58	4.87±0.93[d]	19.76±1.56[c]	11.08±2.84[a]	19.45±1.58[a]
2	6.70 ±1.41	21.26 ±1.4	5.69 ±0.88	17.03±0.95	5.22±0.75[c]	20.27±0.87[b]	9.12±2.16[bc]	19.55±0.95[a]
3	6.89 ±0.63	21.54 ±0.42	6.38 ±1.18	16.97±1.36	6.05±0.65[a]	20.71±0.83[a]	9.52±1.47[b]	17.95±1.36[b]
4	6.95 ±1.03	21.80 ±1.19	5.79 ±0.78	15.87±0.93	5.61±0.88[b]	20.63±0.96[ab]	9.22±2.26[bc]	17.87±0.93[b]
5	6.65 ±1.06	21.10 ±1.05	5.69 ± 1.18	17.23±1.31	5.62±0.57[b]	20.48±0.62[ab]	8.34±3.14[c]	17.48±1.31[b]
6	6.06 ±1.03	20.38 ±0.98	6.28 ±0.49	17.41±1.78	5.74±1.09[ab]	20.48±1.00[ab]	11.08±3.14[a]	19.69±1.78[a]
Mean	**6.60 ±1.02**	**21.13±0.97**	**5.98±0.88**	**16.70±1.32**	**5.52±0.81**	**20.39±0.97**	**9.71±2.55**	**18.67±1.32**
LSD	**1.0037**	**0.9886**	**0.8740**	**1.1149**	**0.3444**	**0.4209**	**1.0713**	**0.5617**

[a] BD = Berry diameter, [b] TSS = Total soluble solids, [β] Means followed by the same letter were not statistically different (P = 0.05), [α] All means of samples from site A were not significantly different (P=0.05).

3.3. Relationship between table grape physiological quality and concentration of Botrytis cinerea at mature stage

Total soluble solids (TSS) were used to determine the correlation between physiological quality and the levels of *Botrytis cinerea* at harvest (Fig. 3A and 3B). A higher Pearson's correlation between TSS and *B. cinerea* was observed in table grapes harvested in site B (0.1313) compared

with site A (-0.0170). 'Crimson Seedless' table grapes are commercially mature at 16.0 % TSS. The results showed that mature berry samples with higher TSS were associated with higher levels of *B. cinerea*. Furthermore, a significantly higher mean concentration of 9.1 copies μL^{-1} of DNA was detected on asymptomatic table grapes harvested at commercial harvest maturity in site A with total soluble solids ranging between 14.5 % and 18.0 %.

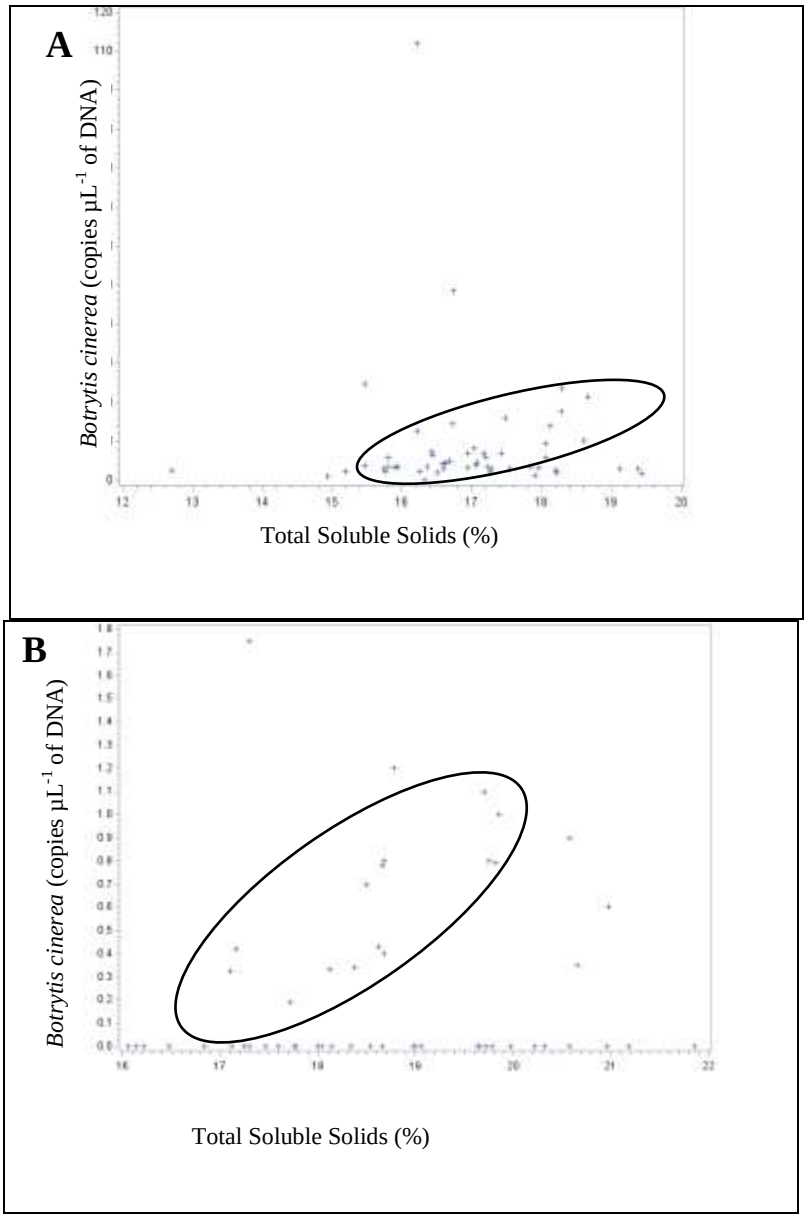

Fig. 3. Correlation between concentrations of *Botrytis cinerea* quantified at commercial harvest time and the total soluble solids of table grapes from two commercial sites (A and B).

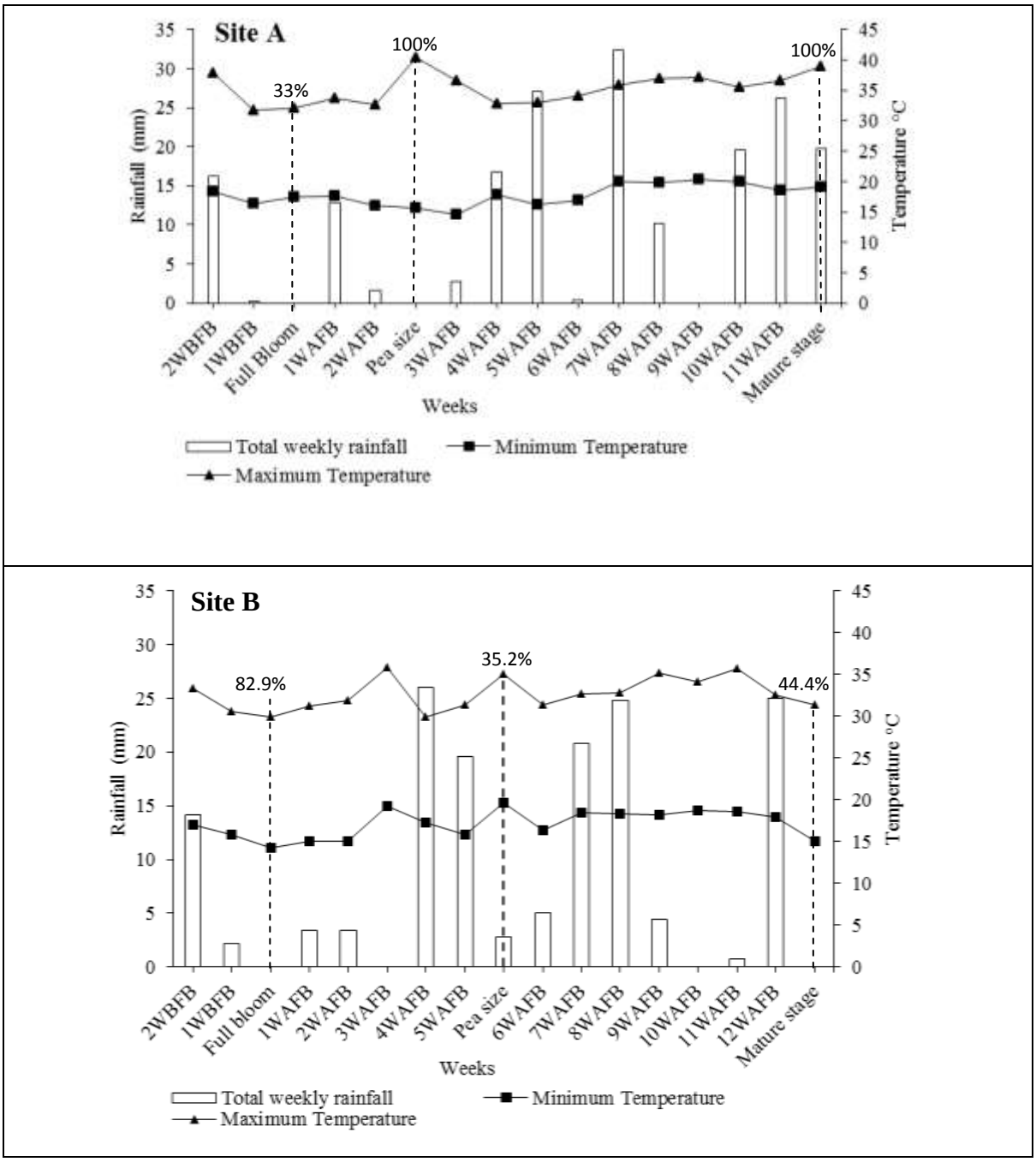

Fig. 4. Accumulated weekly rainfall and average temperatures (from local area meteorological station, South African weather services) from two weeks before full bloom to mature stage. WBFB = weeks before full bloom; WAFB = weeks after full bloom. Dotted lines indicate days of sampling for the three phenological phases. Percentages on top of dotted vertical lines show *Botrytis cinerea* prevalence at the respective stages.

3.4. Site weather conditions during table grape development

Rainfall and temperature data for the two sites was used to define the weather conditions during table grape development (Fig. 4). The weather data showed clear differences between site A and B. Site A received higher total rainfall (166.2 mm) than site B (146.4 mm) during the fruit development phase (full bloom to mature stage). Furthermore, during sampling of the mature stage, 19.8 mm of rainfall was received in site A, while no rainfall was recorded in site B. Site B was dry during specific sampling days of the phenological stages, except during the pea size stage, where only 2.8 mm of rainfall was recorded. A peak in temperature was noted in both sites during sampling of the pea size stage. In Site A, temperature reached 40^{o}C while at 35^{o}C in site B.

4. Discussion

The fresh produce industry, which include table grapes, incur severe losses due to postharvest decay caused by *B. cinerea* during storage and marketing (Anonymous, 2015). Application of pesticides at postharvest is regulated by strict legislations due to food and environmental safety concerns (Romanazzi et al., 2016). With respect to that, control or management of the disease at postharvest is limited to a few alternative strategies that are safe (Servili et al., 2017) and effective at commercial production (Romanazzi et al., 2016). Thus, proper monitoring and early diagnostic of the pathogen on asymptomatic tissue at preharvest is important for disease management to reduce postharvest losses caused by gray mold in table grapes. This would allow selection of vineyards or bunches that can be safely stored or transported for longer duration and help reduce postharvest decay. In this study, we investigated

the level of *B. cinerea* infection at different phenological stages and agro-climatic sites of table grape production.

Results of our study indicate that *B. cinerea* was detected on asymptomatic flowers, pea size and fully mature berries of table grapes using the ddPCR technology but not detected with simultaneously used culture techniques, air samples included (data not shown). Previous studies using culture techniques on blueberries reported similar findings (Rivera et al., 2013). This was attributed to the low sensitivity of conventional culture methods (Sanzani et al., 2014), and possibly the slow growing nature of the fungus on agar, that allow easy competition by the presence of large populations of other rapidly growing fungal communities.

The findings of this study revealed contrasting trends in levels of infection by *B. cinerea* between the two agro-climatic sites. In site B, where higher annual rainfall is received compared to site A, full bloom was the most susceptible phenological stage to *B. cinerea*, a result which has also been noted in previous studies (Nair et al., 1995; Esterio et al., 2011; Rivera et al., 2013). This was evident as the highest levels of *B. cinerea* were noted at full bloom compared to the pea size and mature stages. The levels of the pathogen decreased at pea size and further reduced at mature berry stage. On the contrary, at site A, the lower annual rainfall receiving area, the level of infection increased from flowering to pea size and mature berries. These contrasting results can be explained by temperature and humidity combinations reported previously. Ciliberti et al. (2016) found a higher disease incidence on berries at 90 % relative humidity compared with 100 or 80 % at optimal temperature of 20^{o}C. Esterio et al. (2011) recorded varying infection levels at different phenological stages in three locations. Their results show a decrease in the infection level from veraison to mature berry stages, whereas in the other two locations, the opposite was noted.

Moreover, the differences in climatic conditions could have contributed to the level of *B. cinerea* inoculum between the two sampling areas. An increased concentration of *B. cinerea* from pea size to the mature stage was observed on samples from site A. This was attributed to humid conditions that prevailed prior to sampling, as was also confirmed in a previous study (Blanco et al., 2006). In contrast, the mature berry stage from site B had a lower concentration of *B. cinerea* compared to the pea size. Mature berries from site B, remained at significantly lower concentrations of *B. cinerea* compared to samples from site A. This was partly attributed to the typically dry growing season, which prevailed during the course of the study. Therefore, although recorded average temperature (23.5°C) at this site (B) was closer to the optimum for *B. cinerea* (Steel et al., 2011), low moisture around harvest time was not conducive for the pathogen to proliferate (Williamson et al., 1995). Our results revealed a higher concentration of *B. cinerea* on samples collected from vines located towards the centre of the vineyard than those on the periphery. A conducive microclimate within the vineyard and exposure to sunlight providing a 'sterilizing' effect on peripheral grape bunches have been recently reported to promote and limit pathogen proliferation (Carmichael et al., 2017), respectively. This is a view supported by results of our current report because microclimate manipulation can directly or indirectly affect pathogen growth.

Our results showed that phenological stages with an increased prevalence of *B. cinerea* in asymptomatic flowers and berries were associated with a higher concentration of the pathogen in the samples. Furthermore, a link between *B. cinerea* inoculum at mature berry stage and symptom development after cold storage was observed (Unpublished data). This supports previous work that associated higher incidence of berry infection with faster sporulation of *B. cinerea* (Ciliberti et al., 2016). The association between pathogen inoculum level at harvest and

the development of *B. cinerea* after cold storage indicate the significance of preharvest pathogen detection in order to reduce postharvest loses.

Despite differences in the concentrations of *B. cinerea* between the two sites at harvest, there was a direct correlation between pathogen levels and physiological quality (TSS) of the berries. The TSS is an industry maturity indicator for 'Crimson Seedless" table grapes. At both sites, higher concentration of *B. cinerea* was associated with increasing TSS levels in mature berry samples (data not shown). Thus berries with TSS above 18 % had the highest concentration of *B. cinerea*. Therefore, susceptibility of the berries to pathogen incidence is enhanced during the ripening process. This is due to a decrease in phenolic compounds and organic acids in berries as well as other deciduous fruit (Padgett and Morrison, 1990; Deytieux-Belleau et al., 2009; Harshman et al., 2014; Jia et al., 2016).

Accurate detection of *B. cinerea* early in the season is therefore essential to reduce postharvest losses. This can contribute to improved disease control strategies and developing a more target approach towards reducing postharvest losses. In our study, *B. cinerea* was detected at pea size, a phenological stage where the content of phenols and organic acids is high (Nunan et al., 1998). This is significant because during pea size growth stage, pathogen proliferation is compromised (Harshman et al., 2014; Jia et al., 2016), further confirming the sensitivity of the ddPCR, as shown in previous work (Dreo et al., 2014). It is also important to note that pyrosequencing analysis of the same samples used in our present study did not detect *B. cinerea* (Carmichael et al., 2017), showing that ddPCR was more sensitive in this regard.

The use of stand-alone technologies and applications to control grey mold is not effective at commercial production level (Romanazzi et al., 2016) hence a multidimensional approach is necessary. Effectively, this means that any future method has to include a sensitive pathogen

diagnostic tool such as the ddPCR. This technique can also be a useful tool for researchers to precisely detect and quantify DNA targets of interest in other organisms or plant material. However, future studies should focus on the economic threshold of *B. cinerea* concentrations in vineyards. The method can be used to determine when control measures should ideally be applied at the different phenological stages to ensure more effective disease management.

5. Conclusion

The results of this study provide valuable information on monitoring of *B. cinerea* natural inoculum early during berry development of asymptomatic samples. Such information is of significance in disease prediction modelling, justification of fungicide sprays, programming and planning of harvest dates, storage, packaging conditions and market destinations. This study provides valuable evidence to develop a more effective inoculum monitoring system for asymptomatic grape tissues at a preharvest stage. Determining early preharvest inoculum and prevalence could assist in making informed market decisions, thereby directing consignments to less sensitive markets, based on the level of inoculum present at the mature berry stage.

Conflict of interest

The authors declare no conflict of interest.

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