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RESEARCH PAPERS - 10TH SPECIAL ISSUE ON GRAPEVINE TRUNK DISEASES

Temporal conidial dispersal pattern of Botryosphaeriaceae species in table-grape vineyards in Northeastern Brazil

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Summary. A field experiment was conducted between mid-August 2016 and mid-October 2017 in four table-grape vineyards in the Siriji Valley, Pernambuco State (Northeastern Brazil), to study the conidial dispersal dynamics of Botryosphaeriaceae fungi, causing grapevine trunk diseases (GTDs). Conidial dispersal was assessed by exposing microscope slides coated with Vaseline close to symptomatic plants and pruning debris. The slides were replaced every 2 weeks for a total of 30 sampling periods. Conidia of the genera *Diplodia*, *Lasiodiplodia* and *Neofusicoccum* were enumerated based on morphological characters. Conidia were collected from all four table-grape vineyards, confirming that these fungi are present as aerial inoculum and could be associated with GTDs in the region. Conidia of *Diplodia* and *Lasiodiplodia* were present in all the sampling periods, and those of *Lasiodiplodia* were the most abundant. Conidia of *Neofusicoccum* were found less frequently, and in less numbers than the other genera. Significant correlation between the number of conidia sampled and the amount of rain was observed for *Diplodia* only. Greater numbers of conidia were collected from pruning debris than from symptomatic plants. For *Diplodia* and *Lasiodiplodia*, the numbers of conidia gradually increased in September, increased sharply between March and June, and then decreased. These dynamics were described by a logistic equation, with hydro-thermal time (i.e., a combination of degree-days and relative humidity) as the independent variable ($R^2 > 0.998$).

Keywords: spore traps, *Vitis vinifera*, tropical climate, hydro-thermal time.

Introduction

Viticulture is an important agricultural sector worldwide. In Brazil, the total grape production in 2016 was 1,499,353 t, distributed between table-grapes (48%) and wine-grapes (52%) (De Mello, 2017). The Siriji Valley, located in the northeastern region of Brazil (Pernambuco state), is one of the main areas of table-grape production in this country. The Siriji Valley mainly produces table-grape cv. Isabel, with areas of production characterized by family farms. The valley has a tropical climate (Aw in the Koppen-Geiger climate classification, as indicated by Peel *et al.* (2007)) with an average rainfall of 1075 mm per year and warm temperatures (yearly average of 26ºC) (Tavares and Lima, 2009).

Grapevine trunk diseases (GTDs), caused by several fungal species, are one of the main limiting factors for productivity and longevity of vines (Gramaje *et al.*, 2018; Mondello *et al.*, 2018). Botryosphaeria dieback, caused by Ascomycete fungi in the Botryosphaeriaceae (Philips *et al.*, 2013; Yang *et al.*, 2017), has emerged as one of the most damaging GTDs worldwide (Úrbez-Torres, 2011), because of: i) adaptability to different climatic conditions (Amponsah *et al.*, 2009;

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Kuntzmann *et al.*, 2009), ii) survival in pruning debris and dead plant material (Elena and Luque, 2016), and iii) prolific inoculum production (Van Niekerk *et al*., 2010; Valencia *et al*., 2015).

Symptoms of Botryosphaeria dieback in grapevines include foliar discolouration, fruit rot, dead canes and cordons, vascular necrosis and streaking, perennial cankers, and plant dieback (Amponsah *et al.*, 2011; Úrbez-Torres, 2011; Dissanayake *et al*., 2015; Gramaje *et al*., 2018). In Brazil, Botryospaheriaceae were first associated with grapevines affected by GTDs by Paradela *et al.* (1992) in the '90s. *Lasiodiplodia* and *Neofusicoccum* were frequently associated with GTDs in nurseries and vineyards (Batista *et al*., 2010; Correia *et al*., 2013; Correia *et al*., 2016; Garrido *et al*., 2017).

In the last decades, the taxonomy of the fungal taxa associated with GTDs, and the etiology of these diseases, have been studied in detail (van Niekerk *et al*., 2004; Yang *et al*., 2017). It has been suggested that these fungi overwinter as pycnidia and/or perithecia embedded in the vine bark or on the surfaces of dead grapevine wood. Therefore, the permanence of pruning debris above the vineyard soil can be an important source of inoculum for subsequent seasons (Elena and Luque, 2016). Conidia and/or ascospores are mainly released by these fruiting bodies during rain events or under moist conditions, with temperatures above freezing (Úrbez-Torres, 2011; Gramaje *et al*., 2018). Infection of grapevine tissues can occur through natural openings, although it is mainly associated with the existence of pruning wounds or weak graft unions (Úrbez-Torres, 2011). Pruning shears can contribute to spread of GTD pathogens in affected vineyards (Agustí-Brisach *et al.*, 2015).

The dynamics of Botryosphaeriaceae spores in vineyard environments have been studied recently because of their relevance for disease management (Amponsah *et al*., 2009; Kuntzmann *et al*., 2009; Úrbez-Torres *et al*., 2010; Van Niekerk *et al*., 2010; Baskarathevan *et al*., 2013; Valencia *et al.*, 2015). The temporal dispersal patterns of these spores varied among studies, probably due to the geographical location, the fungal species, and the spore sampling methods (Gramaje *et al*., 2018). Since all these studies have been conducted in regions with temperate climates, no information exists about the dispersal of Botryosphaeriaceae spores under tropical conditions. Moreover, the dispersal pattern of conidia from pruning debris has not been considered in previous studies.

The objectives of the present research were: i) describe the temporal dynamics of conidial dispersal of Botryosphaeriaceae in four table-grape vineyards in the Siriji Valley, characterized by tropical climate, considering symptomatic plants and pruning debris as inoculum sources; ii) examine environmental variables associated with conidial dispersal; and iii) develop equations for predicting the temporal dispersal pattern of Botryosphaeriaceae spores under tropical climate conditions, which could be used to identify the periods with high risk of spore dispersal.

Materials and methods

Vineyards

The study was conducted in four table-grape vineyards located in St Vicente Ferrer, in the tropical region (Siriji Valley) of Pernambuco state (Brazil). Vineyard one (7°34′53.4″S, 35°31′01.2″W), area 1.2 ha, was 14 years old; Vineyard two (7°35′27.2″S, 35°31′35.8″W), area 0.5 ha, was 18 years old; Vineyard three (7°35′21.3″S, 35°31′20.9″W), area 0.8 ha, was 9 years old; and Vineyard four (7°.34′.53.7′S, 35°30′05.3″W), area 1.4 ha, was 15 years old. All these vineyards were affected by GTDs at different levels of incidence (Vineyard 1, 22% of plants affected; Vineyard 2, 13%; Vineyard three, 23%; Vineyard 4, 98%). All four vineyards were planted with cv. Isabel on double cordon system, and cultural practices (irrigation, fertilizer plant protection applications) were performed following the usual practice. No fungicides were applied to control GTDs.

Data of temperature (T, °C), rainfall (R, mm), and relative humidity (RH, %) were obtained from the Agência Pernambucana de Agua e Clima (Apac), responsible of the agrometeorological service in the region. Hourly records of T and RH were obtained from two weather stations situated in Carpina (7°50′57.3″S 35°14′19.5″W) and Goiana (7°38′24.1″S 34°57′21.4″W), located in opposite directions, respectively 42 km north-east and 62 km south-east from the vineyards. All three locations (the vineyards and the two weather stations) are in a flat area of the Siriji Valley. A preliminary analysis of the weather data recorded at Goiana (y) and Carpina (x) showed close linear relationship for T and RH, the regression equations being, for T, $y = 1.009x$ ($R^2 = 0.997$; $P < 0.001$) and for RH, $y = 1.030x$ ($R^2 = 0.994$; $P < 0.001$). Therefore, the weather data measured at both stations was con-

sidered representative of the vineyards under study. Thus, daily averages of T and RH were calculated using the values from the two stations. Daily rainfall data were obtained from Apac for St Vicente Ferrer, the location of the four vineyards.

Conidial dispersal from symptomatic plants and pruning debris

In each vineyard, conidial dispersal was evaluated by exposing spore traps between 22 August 2016 and 17 October 2017, a period comprising two grape harvests. Spore traps were microscope slides coated on one side with petroleum gel (Vaseline). Spore traps were located, (i) in symptomatic plants or (ii) close to pruning debris. For (i), ten plants with the typical Botryosphaeria dieback symptoms were selected, and one microscope slide per plant was attached (oriented horizontally) to the cane with a clip, the gel side upward. For (ii), pruning debris were collected at the beginning of the experiment and arranged to form three piles (diam. 50 cm and height 30 cm), each pile with canes from 50 symptomatic vines. Two spore trap devices were located at 10 cm from the pruning debris and 30 cm above the soil (supported by a metal stake). Each spore -trap device comprised two microscope slides inserted into slots on a polystyrene slab; the slides were in parallel, with the adhesive gel side oriented towards the pile of pruning debris. The slides were covered by a Petri dish to avoid washing by rain (González-Domínguez *et al*., 2014).

Microscope slides were replaced every 2 weeks, commencing on 5 September 2016, for a total of 30 sampling periods. Microscope slides were brought to the laboratory, and each prepared for microscope observation by adding lactophenol cotton blue to the gel side and a cover slip. Conidia of *Diplodia*, *Lasiodiplodia* and *Neofusicoccum* were identified based on the morphological characteristics described by Philips *et al.* (2013), and were enumerated using a compound microscope (40× magnification). All the cover slip area (400 mm^2) of each slide was considered. The ascospores of these genera were not considered because their sexual stages have rarely been found in grapevines (Philips *et al*., 2013, Úrbez-Torres *et al*., 2013), so that conidia are considered to be the principal sources of infection for these fungi (Úrbez-Torres *et al*., 2013). The numbers of conidia were expressed as the cumulative numbers collected over a 2 week period per cm^2 of trap surface. In aggregate, there were 1,200 microscope slides on symptomatic plants and 1,440 near pruning debris piles.

At each sampling period, the growth stage of vines was assessed using the scale described by Lorenz *et al.* (1995).

Data analyses

All data analyses were performed using the software R (v 3.4.0; R CoreTeam, 2014). In all the analyses, the four vineyards were considered as replicates.

The relationship between the amount of rainfall (mm) and the numbers of Botryosphaeriaceae conidia found on the microscope slides (for each genus and inoculum source) was evaluated through the nonparametric Spearman rank correlation coefficients (using the function *cor.test* from the 'stats' package).

The cumulative numbers of conidia at different times were expressed as proportions of the total seasonal conidia (PSC) for each vineyard, genus, and inoculum source. The averages and the standard errors for the different vineyards were then calculated for the genera *Diplodia* and *Lasiodiplodia*, and for both inoculum sources (symptomatic plants and pruning debris). *Neofusicoccum* was not considered in this analysis because of the low numbers of these conidia found during the study.

Average PSC values were regressed against time (t = 1 is the first day in which the microscope slides were exposed), using a logistic equation in the following form (Campbell and Madden, 1990):

$$y = 1/(1 + (a \times \exp(-b \times t))) \quad (1)$$

where: y is the PSC; a is the equation parameter accounting for the length of the lag phase of the S-shaped curve; b is the rate parameter; and t is time. The equations were fitted to the data using the *nls* function of the 'stats' package.

To assess the effects of the environment on the dynamics of conidial dispersal, PSC values were regressed against the thermal or hydro-thermal time (Lovell *et al*., 2004). PSC was calculated as the sum of conidia from symptomatic plants and pruning debris, and this value was averaged between the vineyards. Average PSC values for *Diplodia* and *Lasiodiplodia* was also calculated. Time was expressed as a function of thermal or hydro-thermal time in three different forms: (i) the combination of degree-days and RH (DD-RH), (ii) temperature dependent mycelial

growth rate (MGR), and (iii) the combination of MGR and RH (MGR-RH). For (i), the daily values of T and RH were accumulated during the experimental period in the form: ∑TxRH/1000. For (ii), daily values of T were accumulated as a function of mycelial growth rate (MGR). The MGR was calculated using data from a laboratory experiment developed by Netto *et al.* (2017) with four isolates of *L. theobromae* from the Pernambuco state (Brazil). Supplementary material S1 briefly describes the experiment developed by Netto *et al.* (2017) and the non-linear model constructed for this research. For (iii), daily values of T and RH were accumulated in the form: ∑MGR×RH/100.

In a preliminary analysis, logistic and Gompertz equations were fitted to the PSC values, using the thermal or hydro-thermal time parameters as independent variables (i.e., DD-RH, MGR, and MGR-RH) (Madden *et al.*, 2007). Logistic equations were selected because of their better goodness of fit. Goodness of fit of the different equations was assessed using the adjusted R^2, the magnitude of the standard error of the parameters, the coefficient of residual mass (CRM) and the concordance correlation coefficient (CCC) (Nash and Sutcliffe, 1970; Lin, 1989; Madden *et al.*, 2007). CRM is a measure of the tendency of the equation to over- or under-estimate the observed values (a negative CRM indicates a tendency of the model toward over-estimation). The CCC is the product of two terms: the Pearson correlation coefficient and the coefficient *Cb*, which indicates the difference between the best fitting line and the perfect agreement line (CCC = 1 indicates perfect agreement). CCC was calculated using the *epi.ccc* function of the 'epi.R' package.

Results

Meteorological conditions

A summary of the environmental conditions registered during the experiment is shown in Figure 1. The daily temperature showed low variability, with an average of 26.4ºC and fluctuations < 5°C (Figure 1A). Daily relative humidity was always > 70% (Figure 1B). The rainfall accumulated during the 2 week sampling periods showed high variability, with an average rainfall of 46.6 mm and a maximum of 167.5 mm. No rainfall was recorded in four of the 30 sampling periods, and accumulated rainfall was less than 40 mm in 17 periods. In ten periods, > 60 mm rainfall was recorded, and in four periods, > 100 mm rainfall occurred (Figure 1, C and D).

Conidia from symptomatic plants and pruning debris

Conidia of *Diplodia*, *Lasiodiplodia* and *Neofusicoccum* were found on microscope slides placed close to both inoculum sources, symptomatic plants and pruning debris. No other Botryosphaeriaceae spores (i.e., ascospores or conidia of other genera of the family) were found. A total of 3.72×10^6 conidia cm^{-2} were enumerated; 91.0% of the conidia were of *Lasiodiplodia*, 6.5% were of *Diplodia* and 2.5% were of *Neofusicoccum*. Conidia of *Diplodia* and *Lasiodiplodia* were recorded from all the 30 sampling periods, whereas *Neofusicoccum* was observed in 22 of the 30 sampling periods from symptomatic plants, and 23 of the 30 sampling periods from pruning debris (Figure 2). Of total conidia, 18.8% were found on microscope slides on symptomatic plants and 81.3% on slides close to

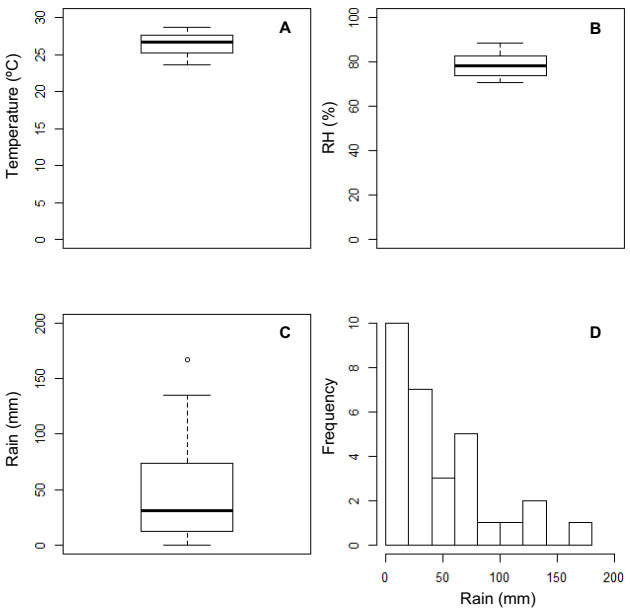

Figure 1. Boxplots of the distributions of temperature (T, ºC) (A), relative humidity (RH, %) (B), accumulated rainfall (in mm) (C), and frequency distribution of accumulated rainfall (in mm) (D), registered during 30 2-week periods in which the dispersal of *Diplodia*, *Lasiodiplodia*, and *Neofusicoccum* conidia was studied in four Brazilian vineyards. Boxes include the 2nd and 3rd quartiles of the data, the dotted line is the median, whiskers extend to minimum and maximum values, and points are outliers.

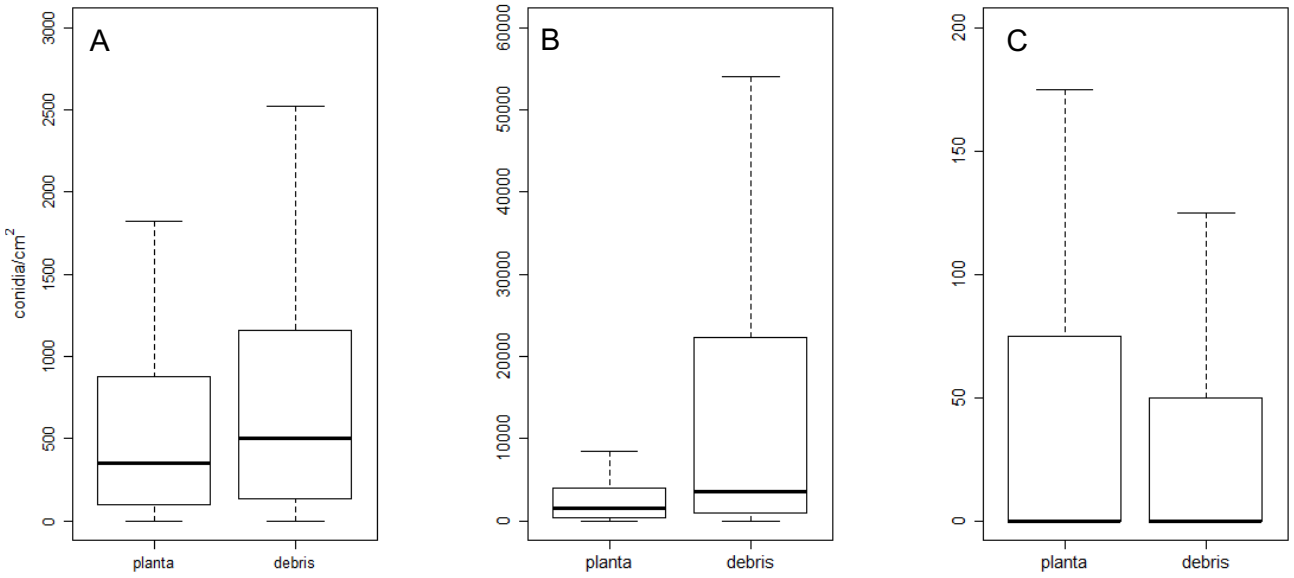

Figure 2. Boxplots of the distributions of the numbers of conidia sampled per cm^2 of microscope slide spore trap surface, from symptomatic plants or pruning debris, found during 30 2-week sampling periods, for *Diplodia* (A), *Lasiodiplodia* (B), or *Neofusicoccum* (C). Boxes include the 2nd and 3rd quartiles, the dotted line is the median, and whiskers extend to minimum and maximum values. Outliers are not shown.

pruning debris. These differences were greater for *Lasiodiplodia* than for the other two genera, with a total of 5.98×10^5 cm^{-2} trapping surface from symptomatic plants, and 8.69×10^4 conidia cm^{-2} from pruning debris (Figure 2).

Temporal dynamics of the conidia

Peaks of numbers of *Diplodia* and *Lasiodiplodia* conidia were mainly found in 2-week periods with high rainfall (Figure 3). On symptomatic plants, the maximum number of *Diplodia* conidia (334 conidia cm^{-2}) was recorded on 28 December 2016, after a 2-week period with a total of 78.9 mm rainfall. Close to debris, the peak of *Diplodia* conidia (413 conidia cm^{-2}) was recorded on 17 May 2017, after a 2-week period with a total of 129.4 mm rainfall (Figure 3, A and B). Positive correlation was found for amounts of rainfall with the numbers of *Diplodia* conidia from symptomatic plants ($r = 0.62$, $P < 0.001$), and with numbers of these conidia from pruning debris ($r = 0.59$, $P < 0.001$).

Few conidia of *Lasiodiplodia* were found at the beginning of the season. Most of these conidia were sampled from February to mid-June (45.1% of the total conidia) from symptomatic plants, and from January to July (95.0%) from pruning debris (Figure 3C).

Conidia of *Neofusicoccum* were sampled from symptomatic plants mainly from mid-October to mid-December 2016. Near pruning debris, most of these conidia were sampled from the 23 August 2017 period, representing > 65% of the total conidia (Figure 3D). No significant correlations were found between rainfall and numbers of *Lasiodiplodia* or *Neofusicoccum* conidia.

When the conidia were expressed as the proportions of the seasonal conidia (PSC), dynamics over time were similar and fitted to logistic regressions, with $R^2 > 0.95$ (Figure 4 and Table 1). The variability in PSC in the different vineyards (standard errors, in Figure 4) was within the 95% confidence intervals of the logistic equations (dashed lines in Figure 4). This confirmed that the logistic equations represent the different vineyards well.

When time was expressed as thermal or hydrothermal time (i.e., DD-RH, MGR, or MGR-RH), the goodness of fit of the logistic equations increased, with $R^2 > 0.98$ and CCC > 0.99 for both *Diplodia* and *Lasiodiplodia* (Table 2); CRM values were low, indicating that the equations did not under- or over-estimate the real data. The fit of the logistic equations to the

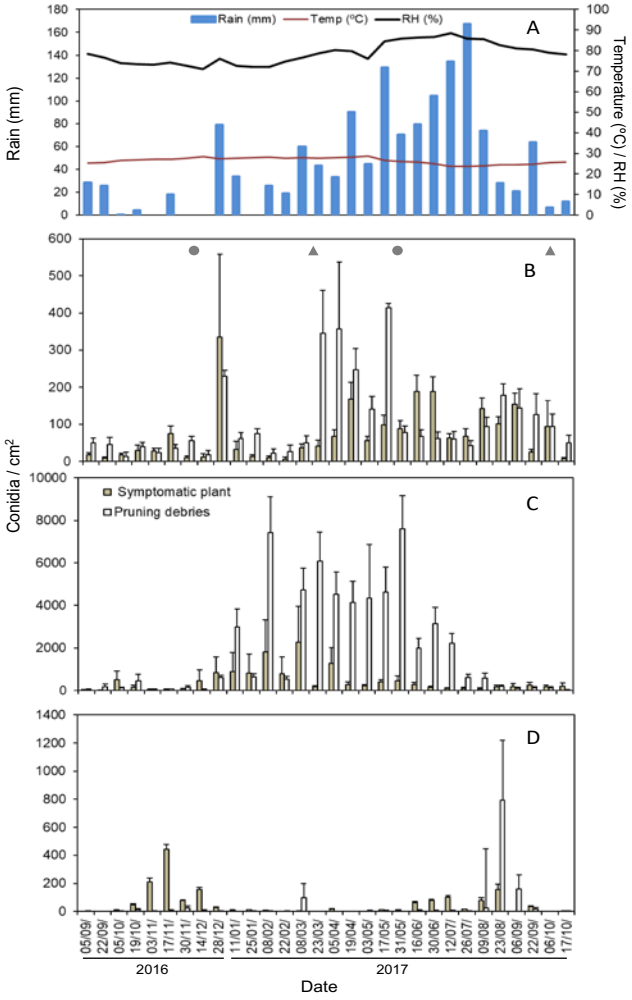

Figure 3. Numbers of conidia of *Diplodia* (B), *Lasiodiplodia* (C), or *Neofusicoccum* (D) sampled per cm^2 of microscope slide spore trap surface, from symptomatic plants or pruning debris, found between 22 August 2016 to 17 October 2017 in four vineyards in St Vicente Ferrer (Brazil); in A, the weather data are also presented. Spore traps were replaced every 2 weeks, commencing from 5 September 2016. Bars are averages for the four vineyards, and whiskers represent the standard errors. In B, grey triangles show the full flowering stage of plants (stage 65; Lorenz *et al.*, 2005) and grey circles show the berries ripe stage (stage 89).

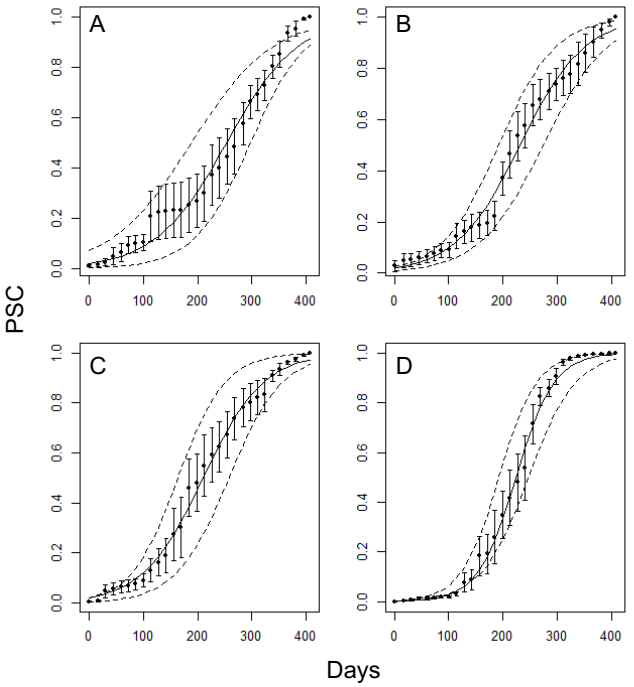

Figure 4. Proportions of the seasonal *Diplodia* conidia (PSC), sampled from symptomatic plants (A) or pruning debris (B), and for *Lasiodiplodia* from symptomatic plants (C) or pruning debris (D), in St Vicente Ferrer (Brazil), between August 2016 and October 2017. Points represent average values for four vineyards and bars represent the standard errors. Lines show the logistic equation fitted to the data (—), and its 95% confidence limits (---) (see Table 1 for parameters of the four equations).

PSC values were also good when data from *Diplodia* and *Lasiodiplodia* were pooled, indicating that the conidial dispersal patterns were similar for the two genera. In general, based on AIC and CRM, the DD-RH better represented the temporal dynamics and should be used to express time. When DD-RH was used, and both genera were considered together, the R^2 and CCC of the model were 0.998, the least value of CRM was obtained (0.007), and the SE of the parameters was low (Table 2).

Discussion

In this research, the temporal dynamics of conidial dispersal of Botryosphaeriaceae fungi (specifically *Diplodia*, *Lasiodiplodia* and *Neofusicoccum*) were investigated by using spore samplers. The samplers were located on symptomatic plants or near pruning debris, in four table-grape vineyards of Northeastern Brazil, under tropical climatic conditions. The tropical climate is characterized by precipitation distributed regularly through each year, and warm temperatures and high relative humidity (Peel *et al.*, 2007). To

Table 1. Estimated parameters of logistic equations used to describe the proportions of seasonal *Diplodia* and *Lasiodiplodia* conidia (PSC) found over time from symptomatic plants or pruning debris in four vineyards in St Vicente Ferrer (Brazil), between August 2016 and October 2017.

Source	Genera	Estimated parameters[a]		R^2
		a	*b*	
Plant	*Diplodia*	7.78 (12.77-300)	0.008 (0.013-0.019)	0.976
	Lasiodiplodia	45.30 (46.59-196.40)	0.018 (0.023-0.002)	0.994
Debris	*Diplodia*	50.81 (41.39-105.60)	0.017 (0.019-0.017)	0.989
	Lasiodiplodia	609.10 (263.50-300.00)	0.028 (0.029-0.023)	0.997

[a] Regression equation is $y = 1/(1+(a \times \exp(-b \times t)))$, where y is the proportion of seasonal conidia (PSC), a and b are the equation parameters, and t is the time (t = 1 is the first day in which the microscope slide spore traps were exposed). Confidence intervals of the estimated parameters are shown in parentheses.

Table 2. Parameters and goodness-of-fit statistics of the equations used to describe the effects of different physiological units on the cumulative numbers of Botryosphaeriaceae conidia sampled from symptomatic grapevine plants or pruning debris, in four vineyards in St Vicente Ferrer (Brazil) between August 2016 and October 2017.

Fungi	Physiological units[a]	Estimated parameters[b]			Goodness-of-fit[c]		
		a	*b*	R^2	CRM	CCC	AIC
Diplodia	DD-RH	48.93 (6.69)	0.07 (0.002)	0.991	0.017	0.996	-117.03
	MGR	75.50 (16.25)	1.97 (0.093)	0.984	0.032	0.991	-98.49
	MGR-RH	58.10 (10.46)	2.45 (0.103)	0.987	0.026	0.993	-104.17
Lasiodiplodia	DD-RH	132.30 (13.64)	0.10 (0.002)	0.998	0.002	0.999	-154.46
	MGR	193.91 (29.94)	2.63 (0.072)	0.997	0.015	0.998	-134.99
	MGR-RH	143.13 (18.29)	3.30 (0.082)	0.997	0.009	0.998	-142.12
Both	DD-RH	71.01 (5.61)	0.08 (0.001)	0.998	0.007	0.998	-157.76
	MGR	108.27 (15.77)	2.24 (0.06)	0.999	0.021	0.997	-128.15
	MGR-RH	81.75 (9.37)	2.79 (0.07)	0.996	0.015	0.998	-137.79

[a] DD-RH (degree-days and RH) were calculated by accumulating the daily values of T and RH during the sampling period; MGR (temperature dependent mycelial growth rate) was calculated by regressing data from a *Lasiodiplodia* spp. laboratory experiment (Netto *et al.*, 2017) as indicated in the supplementary material S1. MGR-RH was calculated by accumulating daily values of MGR and RH.

[b] Regression equation is $y = 1/(1+(a \times \exp(-b \times t)))$, where y is the proportion of seasonal conidia (PSC), a and b are the equation parameters, and t is the time expressed as physiological units. Standard errors of the estimated parameters are shown in parentheses.

[c] R^2, coefficient of determination; CRM, coefficient of residual mass; CCC, concordance correlation coefficient; AIC: Akaike's information criterion.

our knowledge, this is the first study addressing the dispersal of Botryosphaeriaceae conidia under these conditions.

The spore samplers used to study the conidial dispersal were slightly different for symptomatic plants and for pruning debris. In the former case, horizontal microscope slides attached to plants were used, and these are suitable for trapping rain-splashed spores. In the latter case (pruning debris), vertical microscope slides were used, which are suitable for trapping airborne spores (Campbell and Madden, 1990). This difference may have affected the numbers of conidia

trapped by the two spore sampler types. However, the inoculum from infected plants may be mainly splash-borne, and mainly air-borne (i.e., wind-driven, splashing droplets) when produced from pruning debris above the vineyard soil. Thus, the different types of spore samplers used probably reflect this difference.

Conidia of *Diplodia*, *Lasiodiplodia* and *Neofusicoccum* were collected from all four of the table-grape vineyards, confirming that these fungi are associated with GTDs in Northeastern Brazil (Cimmino *et al*., 2017). However, conidia of *Neofusicoccum* were found less frequently, and in lower numbers, than the other two genera, both from symptomatic plants and pruning debris. In this region, the occurrence of *Neofusicoccum* may be less than *Lasiodiplodia* and *Diplodia*. Only recently has the occurrence of *Neofusicoccum* spp. associated with GTDs been reported in Brazil (Correia *et al*., 2013). Similar to the results of the present study, fewer conidia of *Neofusicoccum* than *Diplodia* were trapped in a 2-year experiment conducted in Chile (Valencia *et al.,* 2015). Further studies are required to provide understanding of the implication of *Neofusicoccum* spp. in the development of GTDs in grapevines in Brazil, as well as in other countries.

Lasiodiplodia and *Diplodia* conidia were trapped in all the sampling periods, and in high numbers in the case of *Lasiodiplodia*. The dispersal of both genera was related to rainfall events, as rainfall occurred in 27 of the 30 sampling periods. This result is similar to those from previous vineyard spore-trapping studies, in which Botryosphaeriaceae conidia were captured during and/or following rainfall (Úrbez-Torres *et al*., 2010; Van Niekerk *et al*., 2010; Valencia *et al*., 2015). Studies in South Africa detected conidia of these fungi during or after as little as 0.25 mm rainfall (Van Niekerk *et al*., 2010), and found significant correlations between numbers of conidia and the amounts of rainfall; these correlations were also found by Úrbez-Torres *et al.* (2010) in California. In the present study, statistically significant correlations with rainfall were found for numbers of *Diplodia* conidia, but not for those of *Lasiodiplodia* or *Neofusicoccum*.

Conidia of *Diplodia, Lasiodiplodia* and *Neofusicoccum* were also found in the four periods when no rainfall was recorded. In other pycnidia-producing fungi, moderate to warm temperatures and high relative humidity (> 80%) promote the production of pycnidia, and increase the number of conidia produced per pycnidium (Lalancette *et al*., 2003; Anco *et al*., 2013; Onesti *et al*., 2017). The extrusion of cirri from pycnidia requires free water; but RHs close to 100% can provide sufficient moisture for extrusion (Janex-Favre *et al*., 1993; Onesti *et al*., 2017). In addition, high RHs may contribute to maintaining the gelatinous matrix of the cirri for long periods, favouring the viability of released conidia (Moore and Ostry, 2015). All this information collectively makes it plausible that in tropical climates, with warm temperatures and high RH throughout the year, the dispersal of Botryosphaeriaceae conidia may occur in the absence of rainfall.

To our knowledge, this is the first study that has evaluated dispersal of conidia of Botryosphaeriaceae from pruning debris. A larger number of conidia were trapped from pruning debris than from symptomatic plants, especially for *Lasiodiplodia*. Elena and Luque (2016) found that conidia of *D. seriata* remained viable in debris up to 42 months after pruning, and warned that pruning debris left in vineyards were long-lasting inoculum sources for this pathogen. The present study confirms this finding for *Diplodia* and *Lasiodiplodia*. Therefore, the elimination of pruning debris (by removal or burning) is a key disease management strategy, to reduce the amounts of pathogen inoculum in vineyards (Gramaje *et al*., 2018). The incorporation of pruning debris into the soil after composting is an alternative disease management practice. Lecomte *et al.* (2006) showed that inoculum of *D. seriata*, *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum*, and *Eutypa lata* was eliminated from grapevine wood tissues after composting for 6 months. Petruta *et al.* (2016) also demonstrated the potential of composting vine pruning debris to control *D. seriata*. However, before recommending this practice, further research is required to confirm these results, and to consider other GTD pathogens.

Although the numbers of *Diplodia* and *Lasiodiplodia* conidia differed for the two inoculum sources, the patterns of conidial dispersal were similar for both genera. Dispersal of *Diplodia* and *Lasiodiplodia* conidia increased gradually at the beginning of the experiment (in September), greatly increased in March and June, and then increased at slow rates between August and mid-October. These trends of conidial dispersal was described by logistic equations (as demonstrated by goodness-of-fit), in which the thermal-time was used as the driving variable. The logistic, Gompertz and monomolecular equations have been widely used in analyses of disease progress (Campbell and Madden, 1990), and thermal or hydro-thermal time

have also been widely used (Lovell *et al*., 2004; Rossi *et al*., 2010b; Onesti *et al*., 2018). In the present study, the logistic equations did not over- or under-estimate the dispersal of conidia for both *Diplodia* and *Lasiodiplodia*. Over-estimation or under-estimation of the real data may limit the predictive capacity of conidial dispersal models, which are essential components of epidemiological models (Dewolf and Isard, 2007). Thus, the equations developed here can potentially be used to predict periods of high risk of conidial dispersal of *Diplodia* and *Lasiodiplodia* in vineyards in the tropical region of Northeastern Brazil. Before recommending their use, these equations need to be validated to assess their accuracy (i.e., closeness of predicted to observed values), with data from different years and locations (Rossi *et al*., 2010a). However, these equations are likely to fit well to new, independent data, because of the stability of tropical climates, where the environment is not undergoing major changes.

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