DANIEL WINTER HECK

# FACTORS AFFECTING THE SPATIO-TEMPORAL DYNAMICS OF FUSARIUM WILT OF BANANAS IN BRAZIL

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de *Doctor Scientiae.*

VIÇOSA
MINAS GERAIS – BRASIL
2019

**Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa**

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H448f
2019

Heck, Daniel Winter, 1990-
Factors affecting the spatio-temporal dynamics of Fusarium wilt of bananas in Brazil / Daniel Winter Heck. – Viçosa, MG, 2019.
v, 109 f. : il. (algumas color.) ; 29 cm.

Texto em inglês.
Orientador: Eduardo Seiti Gomide Mizubuti.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Banana - Doenças e pragas. 2. Epidemiologia. 3. Fusarium. I. Universidade Federal de Viçosa. Departamento de Fitopatologia. Programa de Pós-Graduação em Fitopatologia. II. Título.

CDD 22. ed. 634.77294

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APROVADA: 29 de março de 2019.

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## SUMMARY

## ABSTRACT

HECK, Daniel Winter, D.Sc., Universidade Federal de Viçosa, March, 2019. **Factors affecting the spatio-temporal dynamics of Fusarium wilt of bananas in Brazil.** Adviser: Eduardo Seiti Gomide Mizubuti. Co-advisers: Emerson Medeiros Del Ponte and Miguel Angel Dita Rodríguez.

Fusarium wilt (FW), caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is one of the most destructive diseases of bananas. Great yield losses have been recorded for decades. Nevertheless, information about FW epidemiology is scarce. There is no basic information about the disease intensity, spatio-temporal pattern, spread mode and potential factors involved in these processes. This study aims to answer some of the epidemiology gaps to support the design of efficient management strategies. Initially, a survey was carried out in 30 banana fields in the main producing regions of Brazil. In total, 95 ha and more than 100 thousand plants were evaluated. Aggregation of FW was detected by all analytical methods in 43% of the fields. FW was more aggregated in high-input fields. The incidence of FW in banana fields in Brazil is high and is affected predominantly by cultivar, soil physical factors, and field management. The spatio-temporal patterns of FW epidemics were studied in eight plots, totaling 5 ha. All plants were evaluated bimonthly to FW symptoms and spatio-temporal analyses were conducted. The monomolecular model best fit incidence data over time. Aggregated patterns of diseased plants were frequently observed by quadrat-based methods, and the level of aggregation was higher when FW incidence was also high. The clusters of diseased plants were randomly distributed in most plots. The possible aerial dispersal of *Foc* under field conditions was investigated and spores of the pathogen were caught in Burkard spore trap. To investigate whether weevil borer (*Cosmopolites sordidus*; WB) was an effective vector of FW a comparative epidemiology study under field conditions and an association study between *Foc* and WB were performed. Incidence of FW was lower in the field where the population of WB was managed with *Beauveria bassiana* and higher degree of disease aggregation was observed. A great number of *Fusarium* spp. isolates were associated with WB but none was *Foc*. The design of efficient management strategies to mitigate the damage of *Foc* race 1 and the risk of introduction of *Foc* tropical race 4 require solid knowledge of FW epidemiology.

## RESUMO

HECK, Daniel Winter, D.Sc., Universidade Federal de Viçosa, março de 2019. **Fatores que afetam a dinâmica espaço-temporal da Murcha de Fusarium da bananeira no Brasil.** Orientador: Eduardo Seiti Gomide Mizubuti. Coorientadores: Emerson Medeiros Del Ponte e Miguel Angel Dita Rodríguez.

A murcha de Fusarium (MF), causada por *Fusarium oxysporum* f. sp. *cubense* (*Foc*), é uma das doenças mais destrutivas da banana. Grandes perdas de produtividade foram registradas por décadas. Mesmo assim, informações sobre a epidemiologia da MF é escassa. Não há informações básicas sobre a intensidade da doença, padrão espaço-temporal, modo de disseminação e os potenciais fatores envolvidos nestes processos. Este estudo tem por objetivo responder algumas das lacunas da epidemiologia para suportar o desenvolvimento de estratégias de manejo eficientes. Inicialmente, um levantamento foi realizado em 30 campos de banana nas principais regiões produtoras do Brasil. No total, 95 ha e mais de 100 mil plantas foram avaliadas. O padrão agregado da MF foi detectado por todos os métodos analíticos em 43% dos campos. A MF foi mais agregada em campos com alta tecnologia. A incidência da MF nos campos de banana no Brasil foi alta e é afetada predominantemente pela cultivar, fatores físicos do solo e manejo da cultura. O padrão espaço-temporal das epidemias da MF foram estudadas em oito parcelas, totalizando 5 ha. Todas as plantas foram avaliadas bimensalmente para sintomas da MF e análises espaço-temporais foram realizadas. O modelo monomolecular apresentou o melhor ajuste aos dados de incidência. Padrões agregados de plantas doentes foram frequentemente observados pelos métodos baseados em *quadrat* e o nível de agregação foi maior em maiores incidências. Os agrupamentos de plantas doentes estavam aleatoriamente distribuídos na maioria das parcelas. A possível dispersão aérea de *Foc* sob condições de campo foi investigada e esporos do patógeno foram capturados em armadilhas tipo Burkard. Para investigar se o moleque-da-bananeira (*Cosmopolites sordidus*, CS) foi um vetor efetivo da MF, estudos de epidemiologia comparativa em campo e de associação entre *Foc* e CS foram realizados. A incidência da MF foi menor no campo onde a população de CS foi manejada com *Beauveria bassiana* e maior grau de agregação foi observado. Um grande número de isolados de *Fusarium* spp. estava associado com CS, mas nenhum isolado de *Foc* foi confirmado. Sólidos conhecimentos epidemiológicos da MF são

necessários para o desenvolvimento de estratégias de manejo eficientes para mitigar os danos causados pela raça 1 de *Foc* e reduzir o risco de introdução da raça 4 tropical.

## GENERAL INTRODUCTION

Paradoxically, the success of a control practice can compromise the motivation and continuity of the studies of a plant disease and may even challenge the sustainability of a crop in the future. This conundrum is exemplified by what happened to the banana crop in the past half-century. Banana is the world's most important fruit (Faostat 2018) and is affected by one of the most destructive plant pathogens, *Fusarium oxysporum* f. sp. *cubense* (*Foc*) (E. F. Smith), a fungus that causes Fusarium wilt (FW) (Snyder and Hansen 1940; Stover and Simmonds 1987). In the mid-20th Century, large areas cultivated with bananas were devastated by FW. The substitution of 'Gros Michel', the most planted export cultivar at that time, by varieties of the subgroup 'Cavendish' resulted in immediate control of FW. Consequently, after this accomplishment, initiatives to keep investigating and generating basic information about FW lost impetus in most countries. As it commonly happens with human and animal diseases, old threats can re-emerge and cause severe losses (Ploetz 2005; Morens et al. 2004; Sailleau et al. 2017; Couto-Lima et al. 2017). The appearance of Tropical Race 4 (TR4) of *Foc*, which can overcome resistance of the 'Cavendish' bananas is raising high concerns that include the availability of the fruit in the future. Now, that new strategies to manage need to be devised, researchers face the lack of basic epidemiological knowledge of FW.

The intensity of FW in Brazil, its spatial pattern in the field, the temporal dynamics and the potential factors associated with disease increase in time and space remain largely unknown. To date, there are no accurate quantitative data on the intensity of FW in the different banana producing regions in Brazil. It is generally assumed that FW can reach up to 100% incidence in areas cultivated with highly

susceptible cultivars and favorable environmental conditions. These areas are normally abandoned, converted into pasture or cultivated with other crops. In Indonesia, one of the bigger banana producers, FW incidence was an average of 24%, but can reach 100% (Hermanto et al. 2011). In Africa, some fields showed 77% incidence in Ethiopia and, in East and Central Africa 54% of the fields assessed had more than 40% of FW incidence, with the highest values recorded in Tanzania (64%) (Mengesha et al. 2018; Karangwa et al. 2016). These assessments are important to describe the potential damages and impact of FW. Only two studies were conducted to address the spatial pattern of FW under field conditions (Meldrum et al. 2013; Liu et al. 2015). Temporal dynamics studies are even more scarce. Spatial arrangement is an important ecological process associated with plant disease epidemics (Madden et al. 2007) and can be used to devise hypothesis about pathogen dispersal or suggest mechanisms that give rise to them. Initial inoculum may have a direct relationship with the spatial pattern (Ristaino and Gumpertz 2000). Likewise, studies of disease increase in space and time can help us understand how the epidemic develops and how they can be affected by factors related to the: environment (soil, temperature, etc.); host (cultivars, susceptibility, etc.); pathogen (virulence, dispersal, etc.); and anthropogenic actions. The dynamics of FW epidemics can be strongly affected by the way *Foc* is dispersed in the field and this is one of the main gaps in the epidemiology of FW.

The role of wind and insects as dispersal agents remains elusive despite the impact the proper understanding of the role played by these agents may have to the management of FW epidemics. Members of *Fusarium* spp. and *F. oxysporum* were identified in air samples (Schweigkofler et al. 2004; Palmero et al. 2011; Katan et al. 1997; Lin et al. 2013). In some crops, as basil and tomato, *F. oxysporum* f. sp.

*radicis-lycopersici* and *F. oxysporum* f. sp. *basilici*, were able to infect aerial tissues of the hosts (Rekah et al. 2000). *F. oxysporum* f. sp. *cucumerinum* was also detected in air samples in greenhouse condition, but it was not able to establish in wounds (Scarlett et al. 2015). Regarding the contribution of weevil borer (*Cosmopolites sordidus*) as vector of FW much needs to be clarified. *C. sordidus* is one of the main insect pests of banana. *Foc* propagules were identified in the exoskeleton of the insects (Meldrum et al. 2013). Its role as pest and potentially as disease-vector is an important epidemiological issue. The involvement of wind dispersal of *Foc* propagules can also affect the dynamics of FW epidemics and no conclusive study has been conducted so far to test this hypothesis. Wind dispersal of *Foc* can increase the risk of introduction of more virulent variants, such as the Tropical Race 4, in other areas and preventive measures needs to be developed.

The objectives of the present study are translated in the following research questions: (i) What is the intensity of FW in Brazil? (ii) What is the spatial and temporal dynamics of FW in Brazil? (iii) Can *Foc* propagules be dispersed by the wind? Are weevil borers effective vectors of FW? To address these questions, epidemiological studies were conducted and provided valuable information to help develop better strategies for FW management.

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# CHAPTER 1

## Spatial pattern and factors driving the epidemics of Fusarium wilt of banana.

# Spatial pattern and factors driving the epidemics of Fusarium wilt of banana.

**Abstract**: Management of Fusarium wilt (FW) of bananas depends on the knowledge of the dynamics of the disease in time and space. The objectives of this work were to estimate disease intensity, investigate the spatial pattern of FW in banana fields and to measure the association of physical and chemical variables of the soil factors with FW intensity and spatial pattern. Fields planted with Silk (N = 10), Pome (N = 17) or Cavendish (N = 3) banana subgroups distributed in six regions in Brazil, totaling 95 ha, were evaluated. In each field, all plants were inspected and the symptomatic ones were georeferenced. Information about the cultural practices was collected as well as soil and foliar samples from healthy and diseased plants. Incidence maps were constructed and quadrat-based and distance-based methods were used to investigate the spatial pattern of the disease. FW incidence varied from 0.09 to 41.4%. The incidence of FW was higher in Silk fields (median 14.3%). Aggregation of FW was detected by all analytical methods in 43% of the fields (1 of Cavendish, 11 of Pome and 1 of Silk). In the other 17 fields a total of 85 tests were conducted: 62 revealed aggregation and 23 randomness. Overall, aggregation was detected in 85% of the tests. Soil density was associated with higher disease intensity. FW was more aggregated in high-input fields. The incidence of FW in bananas fields in Brazil is high and affected predominantly by cultivar, soil physical factors, and field management.

**Keywords**: *Fusarium oxysporum* f. sp. *cubense*; Panama disease; disease incidence; cultural practices; management.

## INTRODUCTION

Wilted banana plants, probably by a fungal pathogen, were first observed in Australia in 1874 (Bancroft 1876). In 1890, the banana wilt was reported in Cuba, Central America, and was associated to *Fusarium oxysporum* by E. F. Smith (Stover 1962). Currently, Fusarium wilt of banana or Panama disease (FW) is caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) (E. F. Smith) W. C. Snyder and H. N. Hansen (Snyder and Hansen 1940), a soil-borne pathogen (Stover 1962). The movement of infected planting material was responsible for the fast spread of the disease worldwide (Ploetz 2015). This practice is still common among banana farmers, mainly, but not only in systems of subsistence agriculture (Karangwa et al. 2016). Despite the long history of epidemics damaging banana crops worldwide, there are important gaps of knowledge about basic epidemiological features of FW. One such gap is related to the spatial pattern of FW epidemics.

Spatial arrangement is an important ecological process associated with plant disease epidemics (Madden et al. 2007). Spatial pattern is primarily affected by biological and ecological characteristics of the pathogen and of the disease (Campbell and Benson 1994). A direct relationship between the initial inoculum and the spatial pattern can be seeing in some cases (Ristaino and Gumpertz 2000). Spatial pattern analysis can be used to construct a hypothesis about pathogen dispersal or to suggest mechanisms that have originated the observed pattern (Gigot et al. 2017; Macedo et al. 2018). Quantitative information on population dynamics of pathogens (Madden 1989; Macedo et al. 2018), design of experiments in epidemiological research (Madden and Hughes 1995), sampling programs for disease or pathogen monitoring (Madden et al. 1996; Liu et al. 2015), assessment of crop losses in relation to disease intensity (Hughes 1988), and development of management

48 strategies are some of the goals in studies of spatial pattern analysis of plant diseases
49 (Ristaino and Gumpertz 2000).

50 Studies of the spatial pattern of FW on bananas could help understand the
51 processes involved in the dispersal of *Foc* and to set mitigation actions in case of
52 introduction of new variants of the pathogen into an area. To date, only two studies
53 were conducted to analyze the spatial pattern of FW (Liu et al. 2015; Meldrum et al.
54 2013). An aggregated pattern of FW was found in six fields (1334 $m^2$ each)
55 cultivated with Cavendish banana plants in China (Liu et al. 2015). There was a
56 direct relationship between aggregation and FW incidence, i.e., the higher the disease
57 intensity, the higher the aggregation (Liu et al. 2015). FW was also reported to have
58 an aggregated pattern in banana fields in Australia, but scattered diseased plants
59 could also be found in some fields (Meldrum et al. 2013). The authors suggested that
60 weevil borers could have spread FW and contributed to infect plants far from the foci
61 (Meldrum et al. 2013). Another important biological fact to support for the potential
62 aerial dispersal of the pathogen was the presence of sporodochia and hyphal growth
63 in external surface of senescing leaves (Warman and Aitken 2018). If spores could
64 be dispersed by winds and if inoculation can be successful, diseased plants scattered
65 in the field may constitute in a reasonable outcome in addition to the random
66 distribution of inoculum in the soil. Other forms of spore dispersal cannot be ruled
67 out, such as transport by animals, water, soils, substrates and anthropogenic factors
68 (Dita et al. 2018). However, cultural practices are effective to spread FW inside and
69 outside the field. Some practices, such as sharing contaminated planting material and
70 tools with neighbors or when performing cultural practices in asymptomatic, but
71 infected plants, are common practices in banana plantations and may have been

responsible for the introduction of *Foc* tropical race 4 (TR4) from Southeast Asia to Africa or Middle East (Ploetz et al. 2015).

The lack of essential information about the epidemics of FW in bananas, such as the incidence and the spatial pattern of the disease in different conditions, was one of the major constraints to develop more precise management strategies. The objectives of this study can be summarized by the research questions it attempts to address: (i) what is the intensity of FW in Brazil? (ii) what is the predominant spatial pattern of FW epidemics? (iii) which factors are driving the intensity and spatial pattern of the disease?

## MATERIAL AND METHODS

### Fields

Thirty banana fields with records of naturally occurring epidemics of FW were evaluated in Brazil from March 2016 to April 2017. The fields were classified in six groups based on the level of technology adopted and in their geographic region: The Litoral Sul Paulista (LSP; n = 4 fields), São José do Rio Preto and Araçatuba (SJA; n = 8), located in São Paulo state; Vale do Paraíba Paulista and Zona da Mata (VPM; n = 6), located in São Paulo and Minas Gerais states, respectively; Norte de Minas and Vale do São Francisco da Bahia (NMB; n = 5), located in Minas Gerais and Bahia states, respectively; Norte Catarinense (NCA; n = 5), located in Santa Catarina state; and Norte Pioneiro Paranaense (NPP; n = 2), located in Paraná state (Fig 1a). The fields were distributed from latitude 13°14'S to 26°28'S, from longitude 43°21'W to 50°50'W and from altitude 45 to 1150 masl. These states account for 43% of the planted area and 53% of the Brazilian banana production in 2017 (IBGE, 2018). Field size varied from 0.85 to 6.67 ha and were

96 planted with Silk (10 fields; total of 34.7 ha), Pome (17 fields; 51.4 ha) or Cavendish
97 (3 fields; 8.5 ha) banana subgroups.

98 **Assessments**

99 In each field, all banana plants were visually assessed for external symptoms.
100 When external symptoms were observed, such as wilt and yellowing of older leaves,
101 collapse of leaves at the base of petiole, fallen and dried leaves around the
102 pseudostem, wrinkling and distortion of leaf blades or splitting at the base of
103 pseudostem in a plant of the mat, a small cut was made in the pseudostem to observe
104 internal symptoms. Banana plants affected by FW present yellow, reddish brown or
105 black discolorations in vascular tissues. If external and internal symptoms were
106 present in at least one plant of the mat, the whole mat was considered diseased and
107 was georeferenced using a handheld GPS device (GPSMAP® 64, Garmin).
108 Fragments (5 cm of length x 5 cm of width) of pseudostems taken from 5 to 10
109 symptomatic plants from each field were collected to isolate the pathogen and to
110 confirm the presence of *Foc* by morphological (Leslie and Summerell 2008) and
111 molecular methods (Heck et al. *in development*).

112 Two data sets were constructed with the geographic coordinate data: one
113 containing the polygon and the other containing information about diseased plants of
114 each field. These data sets were converted to a text file and imported into R, version
115 3.5.1 (R Core Team 2018). For each field, the two data sets were used for spatial
116 analyses.

117 Information about cultural practices performed by the farmer, such as
118 cultivar, planting density (spacing), age, chemical and organic products used, soil
119 preparation, and the action performed when a diseased plant showing FW symptoms

120 was identified were acquired for the 30 fields. Data of annual mean temperature and
121 precipitation for each field were obtained from the closest climate station and
122 provided by Instituto Nacional de Meteorologia (INMET) of Ministério da
123 Agricultura, Pecuária e Abastecimento (MAPA).

124 In 21 of the 30 fields subsamples of soil ($n$ = 10) were collected at 0.5 m of
125 symptomatic and asymptomatic banana plants. Approximately 0.2 Kg of soil was
126 taken at 20 cm-deep. The subsamples were pooled to compose one sample per field
127 and analyzed for chemical properties (Raij et al. 2001). Organic matter (OM), pH in
128 $CaCl_2$, phosphorous (P), calcium (Ca), magnesium (Mg), potassium (K), base
129 saturation (V%), copper (Cu), iron (Fe), zinc (Zn), and boron (B) were analyzed.

130 Sand, silt, clay, bulk density, and porosity were the physical variables
131 analyzed (Camargo et al. 2009). Eight samples, 4 from symptomatic and 4 from
132 asymptomatic, per field, in 15 fields were analyzed. In the same 15 fields, the
133 nutritional status of the plants was analyzed collecting two composite samples per
134 field. A sample was made of 10 subsamples of banana leaves. A subsample was a
135 fragment of 10 x 10 cm from the central portion of the leaf blade. The concentration
136 of macro (N, P, K, Ca and Mg) and micronutrients (B, Cu, Fe, Mn and Zn) was
137 determined (Bataglia et al. 1983).

138 **Disease intensity**

139 The number of diseased plants ($y_i$) in each field was recorded and the
140 incidence ($\bar{y}$) calculated as $\bar{y} = y_i / n_i$, where $n$ is the estimated number of plants in the
141 $i^{th}$ field. The total number of plants in each field ($n$) was estimated using the area of
142 the polygon and plant spacing (plant distance within and between rows).

## Spatial analysis

Two types of spatial analyses were conducted: a quadrat-based and a distance-based. The polygons and diseased plants data sets were used to produce maps with the spatial distribution of diseased plants.

### *Quadrat-based*

The diseased plant data set was used for quadrat-based analyses, the maps were *quadratized* using the QUADRATCOUNT function of the SPATSTAT package (Baddeley and Turner 2005). The exact number of plants in each quadrat could not be computed because in most fields the rows were not regularly spaced or straight, but it was assumed that the maximum number of plants inside each quadrat was 4 in 2 x 2 to 36 in 6 x 6 quadrat-sizes. The area ($m^2$) of the sampling units varied among fields because different spacing between plants were observed, but the relation between the distance within and between rows was the same. The number of diseased plants in each quadrat was determined and used to perform the spatial analysis.

*Spatial hierarchy*. Initially, four hierarchical levels, 2 x 2 (lowest), 3 x 3, 4 x 4 and 6 x 6 (highest) estimated plants per quadrat were used to characterize the sampling units. The probability that an element is diseased or contains at least one diseased individual was denominated as $p_{low}$ and $p_{high}$, respectively (Madden et al., 2007). The subscript 'low' refers to within-sampling-unit and 'high' the sampling unit scale. The relationship between $p_{low}$ and $p_{high}$ was described as: $p_{high} = 1 - (1 - p_{low})^v$ (Hughes and Gottwald, 1999). The equation can be rewritten as the complementary log-log transformation (CLL) where $v$ is a parameter that can be estimated by the data and is also interpreted as an effective sample size (Madden and

Hughes, 1999; Madden et al., 2007). Values of *v* less than the number of individuals at the lower hierarchical level per sampling unit (highest level), characterize *β*-binomial curves that fall below the binomial curve and suggest aggregation within-sampling-unit. The spatial hierarchy analysis was performed using SPATIAL_HIER from EPIPHY package.

*Dispersion index*. The index of dispersion for binomial data, *D*, was calculated for each field as the ratio of the observed and the estimated variances (Madden and Hughes, 1995). $D < 1$ indicates underdispersed data (regular pattern); $D = 1$ indicates a random pattern and $D > 1$ indicates overdispersed data (aggregated pattern). To test the null hypothesis for dispersion index ($D = 1$) a $\chi^2$ test was performed. The analysis was conducted using the AGG_INDEX function from the EPIPHY package (Gigot 2018).

*Distributions*. The binomial and *β*-binomial distributions were fitted to the disease incidence data for each individual field evaluated using the 3 x 3 quadrat-size. The binomial model suggest a random pattern for binary data, such as incidence data. On the other hand, the *β*-binomial distribution is suitable for overdispersion of these data type. The *β*-binomial and binomial parameters were estimated for all fields studied by maximum likelihood (Sparks et al. 2008). The $\chi^2$ goodness-of-fit test for both distributions and a log-likelihood ratio test (LRS) were used to determine whether the *β*-binomial better fits the observed frequency than the binomial distribution. This analysis was performed using the FIT_TWO_DISTR function from the EPIPHY package.

*Spatial Analysis by Distance IndicEs* (*SADIE*). This method uses the location of the sampling units (i.e. quadrats) and the number of diseased individuals (i.e. mat) inside the unit to analyze the spatial arrangement of the diseased individuals by the

192 distance to regularity ($D_r$). The $I_a$ statistic given by *SADIE* is an overall index of
193 aggregation obtained by the ratio between the distance moved to achieve a regular
194 pattern for the observed data and a theoretical mean to regularity based on random
195 permutations of the individuals among the sampling units (Perry et al. 1999). *SADIE*
196 was performed using an intermediate quadrat-size (3 x 3). The index developed by Li
197 et al. (2012) was computed by the *SADIE* function from EPIPHY package.

198 *Distance-based*

199 *Point process*. The distance-based statistic (Madden, Hughes and Bosch,
200 2007) was used to complement the quadrad-based methods. In this analysis patterns
201 of points were described based on intervals in space among events. The observed
202 distribution, *T* (coordinates x and y of diseased plants), was compared with a predict
203 distribution (expected) of *T* function assuming CSR by Kolmogorov-Smirnov test
204 (Baddeley and Turner 2005). The goodness-of-fit of the point process model was
205 performed using a CDF.TEST function from SPATSTAT package.

206 *Concordance analysis*

207 Concordance analysis was conducted to check for the agreement of the results
208 from the different methods used to study the spatial pattern of FW. The results of the
209 statistical methods were transformed to a categorical data (aggregated or random)
210 and tested by Cohen´s kappa for paired agreement among the methods, and the Fleiss
211 ´s kappa for an overall agreement for all methods used. The analysis was performed
212 using the KAPPA2 and KAPPAM.FLEISS functions from the IRR package for R
213 (Gamer et al. 2012).

214 *Binary power law*

215 The binary power law was used to evaluate the relationship between the
216 observed variance and the corresponding variance on the assumption of a binomial
217 distribution of FW incidence. The classic equation for binary form of Taylor´s power
218 law, $\log_{10}(s^2_y) = \log_{10}(A) + b \log_{10}(s^2_{bin})$, where $s^2_y$ is the observed variance and $s^2_{bin}$ the
219 corresponding variance under binomial distribution, $A$ and $b$ are the linear regression
220 parameters to be estimated. The analysis was performed using an intermediate
221 quadrat-size (3 x 3). A t-test was performed to test the null hypothesis for a binomial
222 distribution: $A = b = 1$.

223 Categorical values referred to different factors, such as cultivars (Cavendish,
224 Pome or Silk), management levels (high, moderate or low) based on cultural
225 practices (herbicide, insecticide, fungicide or lime applications, soil analysis, organic
226 and chemical amendment, defoliation, irrigation and type of seedlings); density of
227 plants (high or low, with < 10 or > 10 $m^2$ per plant, respectively), planting age (< 5 or
228 > 5 years old), and annual mean temperature (low or high temperature, with < 22 or
229 > 22 ºC, respectively) were included in each data set and the parameter estimates of
230 the models were compared by t-test against the null hypothesis and between them.

231 **Factors driving disease intensity and spatial pattern**

232 To understand the main factors related with disease intensity, the annual
233 average temperature, precipitation, soil chemical and physical properties, the macro
234 and micronutrients in leaves, frequency of herbicide, insecticide or fungicide
235 applications were used in a principal component analysis (PCA) using the
236 FACTOMINER package version 1.41 (Husson et al. 2018). To assess the influence

of management practices in the spatial pattern and intensity of FW, a multiple correspondence analysis (MCA) was performed using the MCA function of package FACTOMINER.

## RESULTS

### Disease intensity

Fusarium wilt incidence ranged from 0.1 to 41.4% across the fields with mean and median values of 10.7% and 5%, respectively (Fig 1b, Table 1). The incidence did not differ ($P$ = 0.151) among the regions. Nevertheless, the highest values were observed in fields located in NPP with median incidence of 22.1%, followed by SJA with a median of 14.3%. In other regions the median incidence values were less than 7.9% (Fig 1b).

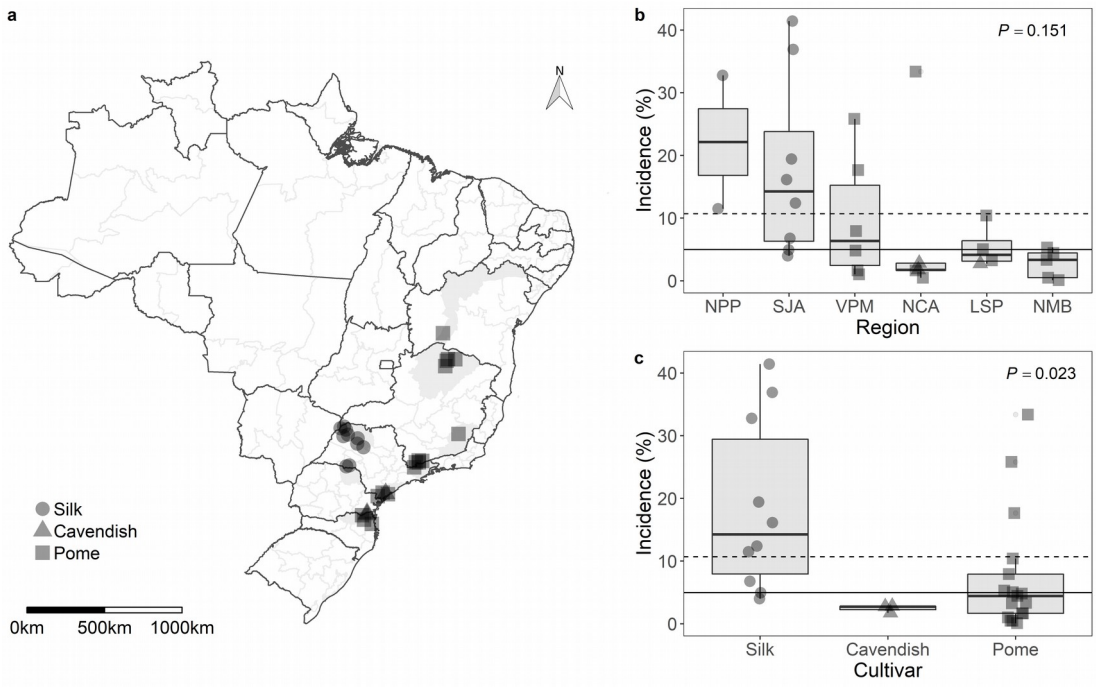



Fig 1. Location of 30 fields in five states of Brazil (a). Mesoregions were shaded by gray and exact location of fields were identified by geometrical shapes that correspond to banana cultivars. Close fields got superimposed and shapes are darker.

Boxplots for incidence of Fusarium wilt on banana fields in different regions from Brazil (b) and for different cultivars (c). The overall mean and median values are represented by dashed and solid lines, respectively.

Table 1. Median value (Med.) for incidence ($\bar{y}$) of Fusarium wilt of bananas and proportions of fields classified as aggregated pattern (Agg.) by the aggregation index ($D$), $\beta$-binomial parameter ($\theta$), log-likelihood ratio statistic (LRS), index of aggregation of the Spatial Analysis by Distance IndicEs (*SADIE*) procedure ($I_a$) and distance-based point process (K-S statistic) relative to different regions [a].

| Region [b] | $\bar{y}$ | $D$ [c] | | $\theta$ [c] | | LRS [c] | $I_a$ [c] | | K-S statistic [d] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Med.[ns] | Med.[ns] | Agg. | Med.[ns] | Agg. | Agg. | Med.[ns] | Agg. | Med. | Agg. |
| LSP | 4.1 | 2.0 | 4/4 | 0.149 | 4/4 | 4/4 | 1.75 | 4/4 | 0.212 ab | 4/4 |
| NCA | 1.8 | 1.5 | 5/5 | 0.076 | 5/5 | 5/5 | 2.15 | 4/5 | 0.126 b | 2/5 |
| NMB | 3.3 | 1.5 | 4/5 | 0.121 | 4/5 | 4/5 | 1.52 | 4/5 | 0.189 ab | 4/5 |
| NPP | 22.1 | 3.1 | 2/2 | 0.357 | 2/2 | 2/2 | 2.22 | 1/2 | 0.212 ab | 2/2 |
| SJA | 14.3 | 2.2 | 8/8 | 0.209 | 6/8 | 8/8 | 1.30 | 3/8 | 0.104 b | 4/8 |
| VPM | 6.4 | 3.5 | 6/6 | 0.455 | 6/6 | 6/6 | 1.83 | 4/6 | 0.337 a | 6/6 |
| Overall | 5.0 | 2.3 | 29/30 | 0.199 | 27/30 | 29/30 | 1.73 | 20/30 | 0.187 | 22/30 |

[a] Spatial statistics were analyzed in 30 fields evaluated in different regions in Brazil. Aggregated pattern was inferred when the null hypothesis of randomness was rejected ($P < 0.05$). To infer about the $\beta$-binomial parameter ($\theta$), the null hypothesis is aggregation ($P > 0.05$).
[b] Litoral Sul Paulista (LSP); Norte Catarinense (NCA); Norte de Minas and Vale do São Francisco da Bahia (NMB); Norte Pioneiro Paranaense (NPP); São José do Rio Preto and Araçatuba (SJA); Vale do Paraíba Paulista and Zona da Mata (VPM).
[c] Quadrat-based statistics with quadrat-size of 3 x 3 plants.
[d] Distance-based statistic.

Differences were observed for incidence among cultivars ($P = 0.023$). The highest values of median incidence were observed for fields planted with Silk (N = 10), 14.3%, a minimum of 4% and maximum of 41.4%. Pome (N = 17) had intermediate median values, 4.4%, and varied from 0.1% to 33.4%. Fields planted

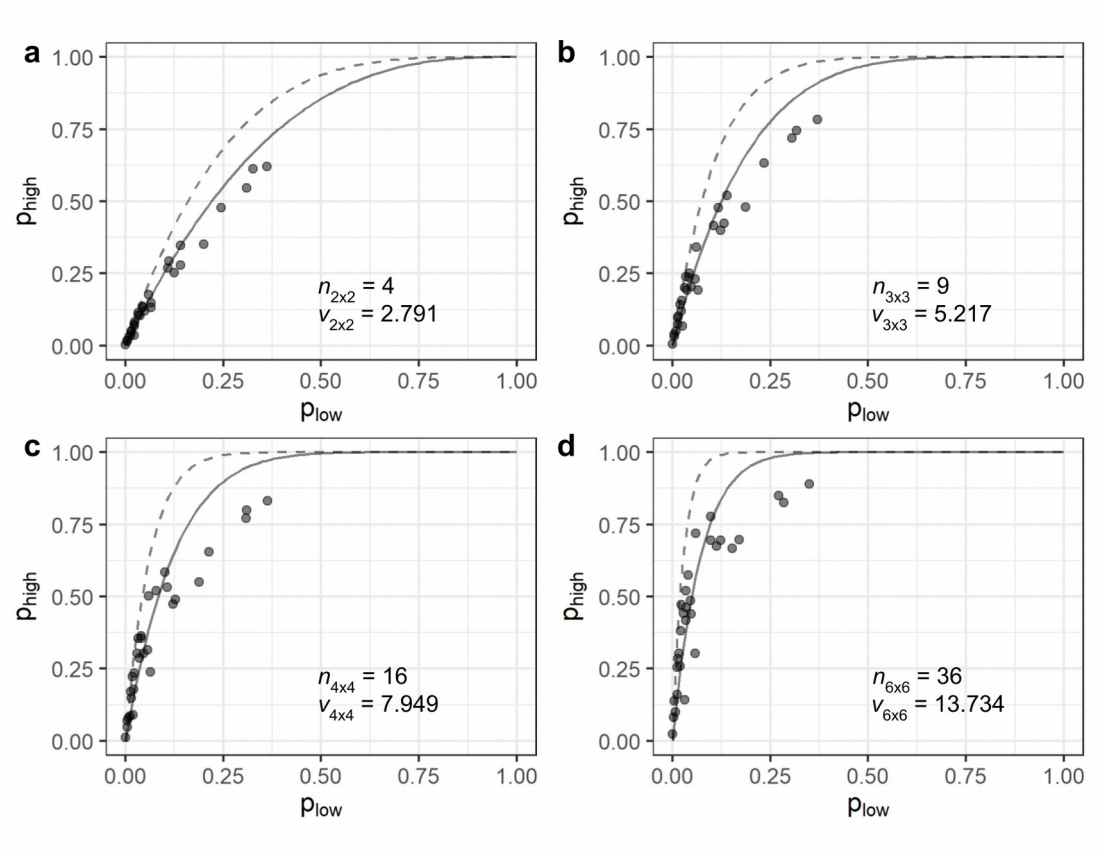
with Cavendish (N = 3) resulted in lowest disease intensity with median of 2.7% and varied from 1.8% to 2.8% (Fig 1c). Incidence on Silk was greater than in Pome ($P$ = 0.039) and in Cavendish ($P$ = 0.005). Pome also differed from Cavendish ($P$ = 0.046).

**Spatial analysis**

*Quadrat-based*

Analyses at the four hierarchical levels resulted in $\beta$-binomial curves that fall under the binomial curves (Fig 2). Values of $v$, interpreted as effective sample size, were lower than the real number of individuals ($n$) for all hierarchical levels ($P <$ 0.001). Effective sample sizes were estimated at $v_{2x2}$ = 2.79 (± 0.12) for the 2 x 2 quadrat-size containing 4 individuals, $v_{3x3}$ = 5.22 (± 0.32) for the quadrat containing 9 individuals, $v_{4x4}$ = 7.95 (± 0.67) for the quadrat with 16 individuals, and $v_{6x6}$ = 13.73 (± 1.48) for the 6 x 6 quadrat-size containing 36 individuals at the highest level (Fig 2). These values of $v$ correspond to 69.8, 57.9, 49.7 and 38.2% of the $n$ in the four hierarchical levels, respectively. Despite that, only an intermediate quadrat-size (3 x 3) was used in further quadrat-based analyses.

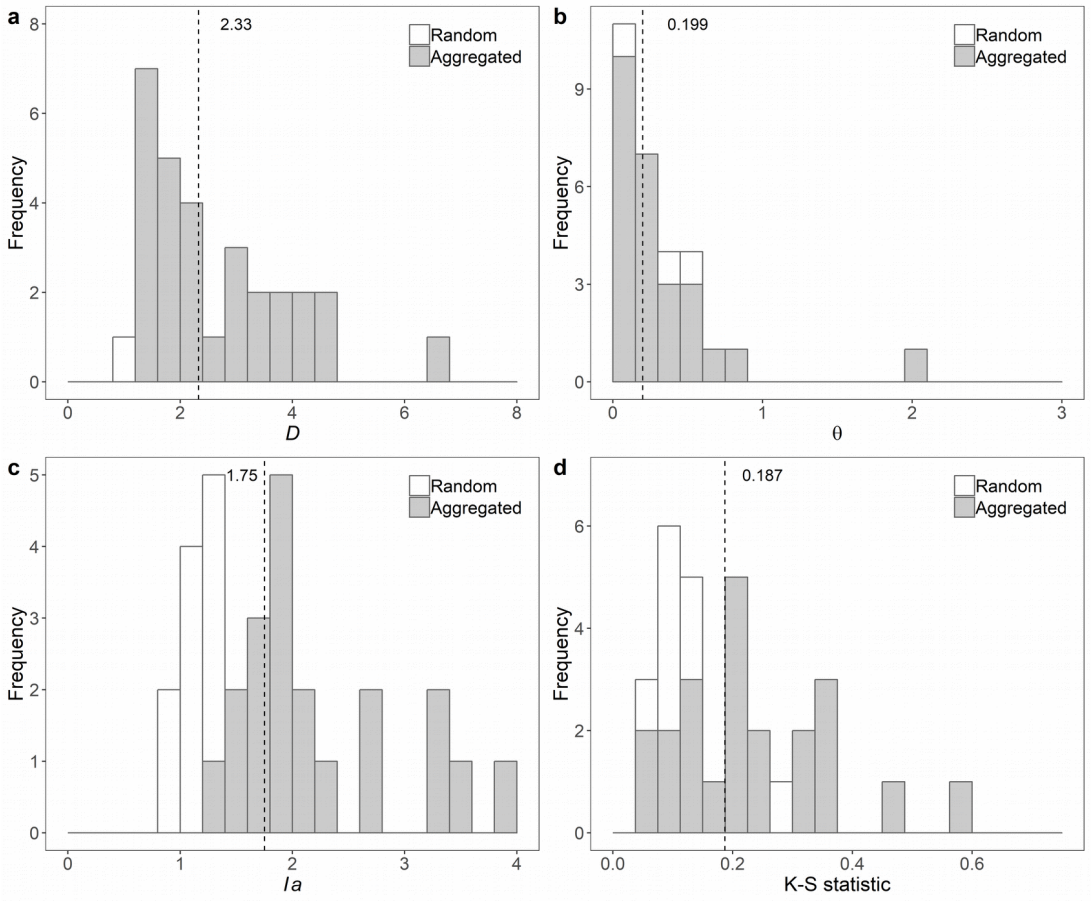
Dispersion index was successfully calculated for the 30 fields using the 3 x 3 quadrat-size. Values of $D$ ranged from 1 to 6.5 with median of 2.3 (Fig 3a). Only one out of 30 fields had a random pattern (Table 1). There were no differences among regions regarding the values of $D$ ($P$ = 0.119) (Table 1).




291 Fig 2. Relationships between the incidences of Fusarium wilt at the 2 x 2 (a); 3 x 3
292 (b); 4 x 4 (c); and 6 x 6 (d) quadrat dimensions collected in 30 banana fields. The
293 sampling unit was the highest and the number of individuals per quadrat at the lowest
294 hierarchical level. Binomial (dashed-lines) and $\beta$-binomial (solid-lines) distributions
295 were fit to the data. The number of individuals ($n$) and effective sample size ($v$)
296 estimated in each level are presented in the graphs.

297 The frequency of diseased plants in quadrats was well described for 90% of
298 the fields by the $\beta$-binomial distribution ($P > 0.05$) (Table 1). For one field (3.3%)
299 the frequency was better described by the binomial distribution (data not shown).
300 The $\theta$ parameter for the $\beta$-binomial distribution ranged from 0.02 to 2.01 (median =
301 0.20) (Fig 3b). There were no differences among regions for the $\theta$ parameter ($P$ =
302 0.26; Table 1). The $\beta$-binomial better described the distribution of diseased plants in

303 the data sets than binomial in 96.7% of the cases ($P < 0.05$) (Table 1). The only case
304 where the LRS was not significant, i.e. the binomial and $\beta$-binomial models could
305 both describe the distribution of diseased plants, was in a field where the FW
306 incidence was very low (0.5%).




307 Fig 3. Histograms of the index of dispersion ($D$; a), $\beta$-binomial parameter ($\theta$) from
308 distribution fitting (b), aggregation index ($I_a$) from Spatial Analysis by Distance
309 IndicEs (SADIE; c) and the Kolmogorov-Smirnov test (K-S statistic) of a point
310 process (d). The frequencies were based on 30 fields assessed for incidence of
311 Fusarium wilt of bananas in different regions. Dashed lines and values represent the
312 median of the statistics. On the spatial statistics graphs the final classification of
313 fields were presented in different colors as random (white) or aggregated (gray).

314 The distance to regularity ($D_r$) computed by SADIE was calculated for all
315 studied fields. The median of the aggregation index ($I_a$) was 1.75 for all fields, but
316 ranged from 0.93 to 3.86 (Fig 3c). Differences among regions were not observed for
317 $I_a$ ($P$ = 0.114; Table 1). Considering the 30 fields in six regions, the random pattern
318 of FW was inferred for 10 (33.3%) fields (Table 1). Five of them were located at
319 SJA, all planted with Silk.

320 *Distance-based*

321 The maximum difference ($D_{K\text{-}S}$) between the observed and expected
322 distributions ranged from 0.064 to 0.566 (median = 0.187) (Fig 3d). The hypothesis
323 of randomness for K-S statistic was rejected for 73.3% of the fields assessed (Table
324 1). Differences among the regions were observed for $D_{K\text{-}S}$ ($P$ = 0.018). The smallest
325 values of $D_{K\text{-}S}$ were observed in SJA and NCA (mean of 0.130) regions where almost
326 all fields inferred as having a random pattern were located. In these regions, there
327 was no evidence to reject the null hypothesis in 50% and 60% of the fields,
328 respectively. Only one field inferred as having a random pattern was located in
329 another region, in NMB. This NMB field had an intermediate value for $D_{K\text{-}S}$ (0.214)
330 and the lowest FW incidence (0.09%).

331 *Concordance analysis*

332 The FW epidemics were inferred to present random or aggregated patterns.
333 Cohen´s Kappa paired agreement was weak or null for most of the spatial statistics
334 used (Table 2). There was weak agreement between the aggregation index and $\beta$-
335 binomial distribution, despite 90% (26/30) of the fields being classified in the same

category (aggregated). The *D* index and LRS agreed ($P < 0.001$) with the highest proportion of fields classified as the same pattern (100%). The *SADIE* index ($I_a$) also shows a weak agreement with K-S statistic ($P = 0.04$), despite 22 of 30 fields presented the same classification, 17 fields as aggregated and 5 as random. For the Fleiss´s Kappa overall agreement among the classification of the spatial analyses the null hypothesis for a random classification was rejected ($P = 0.001$). Agreement could be detected by the test, on which 43.3% (13/30) of the fields presented the aggregated pattern for all spatial statistics (Table 2).

Table 2. Cohen's and Fleiss's Kappa agreement and proportions of fields with the same classification for index of aggregation (*D*), $\beta$-binomial distribution, log-likelihood ratio statistic for distributions (LRS), Spatial Analysis by Distance IndicEs ($I_a$) and Kolmogorov-Smirnov (K-S) statistic for 30 fields assessed to Fusarium wilt incidence in Brazil [a].

| Spatial statistic [b] | $\theta$ | LRS | $I_a$ | K-S statistic |
|---|---|---|---|---|
| *D* | -0.01 (26/30) | **0.03** (30/30) | -0.06 (19/30) | -0.06 (21/30) |
| $\theta$ | | **-0.05**(26/30) | -0.03 (17/30) | -0.03 (19/30) |
| LRS | | | -0.01 (19/30) | -0.01 (21/30) |
| $I_a$ | | | | **0.37** (22/30) |
| Overall [c] | -0.147 ($P = 0.001$) (13/30) | | | |

[a] The fields were classified as aggregated or random pattern according the *P*-values ($P < 0.05$; or $P > 0.05$ for $\beta$-binomial distribution) of the spatial statistic computed. Kappa values in bold differed significantly from 0 (random classification) by z statistic ($P < 0.05$). The proportion of fields classified as the same pattern were in parentheses.

[b] Cohen´s Kappa paired agreement between tests.

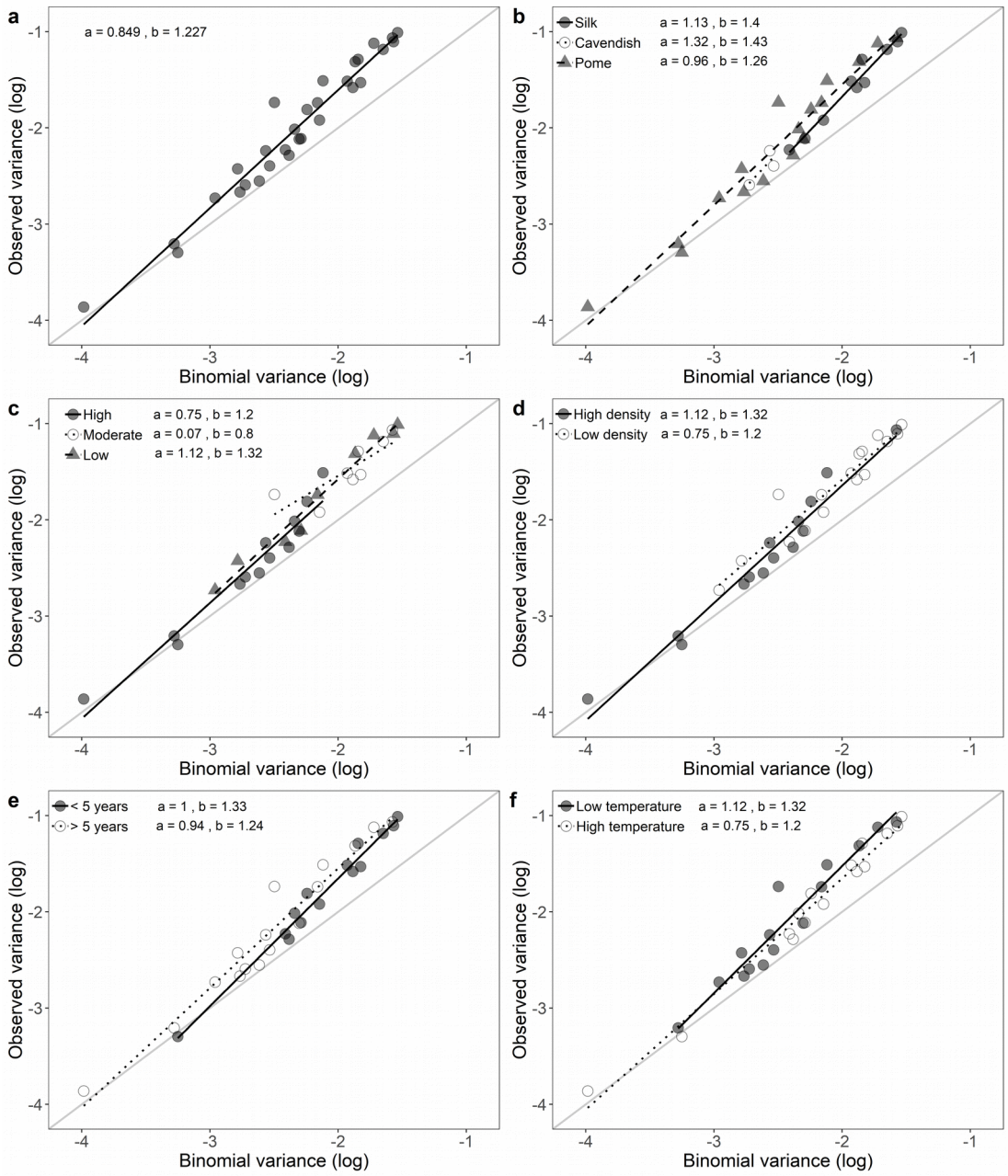
[c] Fleiss´s Kappa overall agreement among the tests.

355 *Binary power law*

356 The relationship between the logarithm of the observed and theoretical
357 variances for binomial data from 30 fields in Brazil was well described by the binary
358 power law ($R^2 = 0.955$) (Fig 4a). Estimates of both parameters of the binary power
359 law, $\log_{10}(A)$ (0.849 ± 0.031) and $b$ (1.227 ± 0.013), were significantly ($P < 0.001$)
360 different from 0 and 1, respectively. As $\log_{10}(A)$ was higher than 0 and $b$ higher than
361 1, in general the disease has an aggregated pattern. Aggregation was directly
362 associated with incidence.

363 The spatial pattern of FW varied according to the cultivar. FW had an
364 aggregated pattern in Silk and Pome fields ($P < 0.001$) (Fig 4b). The estimated
365 parameters from the binary power law differed for Silk and Pome ($P < 0.05$). In
366 general, the level of aggregation was higher and more influenced by incidence in Silk
367 ($\log_{10}(A) = 1.13 \pm 0.17$) than in Pome fields ($\log_{10}(A) = 0.96 \pm 0.19$; $b = 1.25 \pm 0.07$).
368 Cavendish did not differ from Silk and Pome for both parameters ($A$ and $b$).

369 The null hypothesis of random pattern was rejected in fields that used high or
370 low levels of management practices ($P < 0.001$) (Fig 4c). In addition, there were no
371 clear differences among management levels regarding the spatial pattern in fields
372 (Fig 4c). High ($\log_{10}(A) = 0.74 \pm 0.23$; $b = 1.20 \pm 0.08$) and low ($\log_{10}(A) = 0.89 \pm$
373 0.18; $b = 1.23 \pm 0.08$) levels did not differ ($P > 0.05$) between each other for both
374 parameters ($A$ and $b$). However, parameters estimates for the high and low levels of
375 management, both differed for fields classified as of moderate level ($P < 0.003$).
376 There was higher aggregation and lower influence of mean in fields with moderate
377 ($\log_{10}(A) = 0.07 \pm 0.44$; $b = 0.80 \pm 0.23$) than in the high or low levels of
378 management.




379 Fig 4. Relationship between the logarithm of the observed variance ($\log_{10}(s^2)$) and the
380 logarithm of theoretical variance ($\log_{10}(s^2_{bin})$) for incidence data of Fusarium wilt of
381 bananas on 30 fields in Brazil. Linear regression for all of the 30 fields assessed for
382 disease incidence (solid dark line) (a); for Silk ($n$ = 10 fields), Cavendish ($n$ = 3) or
383 Prata ($n$ = 17) cultivars (b); for high ($n$ = 13), intermediate ($n$ = 8) or low ($n$ = 9)
384 levels of management (c); for fields with high density of plants (< 10 $m^2$ plant; $n$ =

14) or low density (> 10 $m^2$ per plant; $n$ = 16) (d); for new plantings (< 5 years old; $n$ = 14) or old plantings (> 5 years old; $n$ = 16) (e); and fields with low temperature (annual average < 22 ºC) or high temperature (annual average > 22 ºC) (f). Binary power law parameters ($A$ and $b$) were presented in figure.

Fields with low or high densities of plants differed ($P$ < 0.001) from random pattern for both parameters and were considered as aggregated by binary power law. The two plant density classes differed from each other for the $b$ parameter ($P$ = 0.01). Fields with high density of plants ($b$ = 1.22 ± 0.06) were more affected by incidence than fields with low plant density ($b$ = 1.14 ± 0.09) (Fig 4d).

When fields were stratified according to age, both, new (< 5 years) and old (> 5 years) plantings, differed from the random pattern for $A$ and $b$ parameters ($P$ < 0.001). Older plantations ($b$ = 1.24 ± 0.08) were less ($P$ = 0.002) affected by the incidence than new ones ($b$ = 1.32 ± 0.06 (Fig 4e).

Aggregation of FW was also detected in fields located in regions with either high or low temperatures ($P$ < 0.001) by binary power law (Fig 4f). Parameters estimates also differed for fields classified between the two classes of temperature ($P$ < 0.001). Fields located in regions with low temperature ($\log_{10}(A)$ = 1.12 ± 0.27; $b$ = 1.32 ± 0.10) had higher degree of aggregation and were more influenced by incidence than plantations in higher temperature regions ($\log_{10}(A)$ = 0.74 ± 0.11; $b$ = 1.20 ± 0.05).

**Factors driving disease intensity and spatial pattern**

The PCA for edaphic variables, management levels and intensity of FW in banana fields explained 73.8% of the total variance (Fig 5a). The first component

explained 49.6% of the total variance, the significant factors with higher contribution were macro (N, K, Ca, Mg, S, K:N and Ca:N) and micronutrients in leaves (B, Cu and Fe), and the soil chemical (P and K) and physical (silt and soil resistance) properties. The second component explained 24.2% of the variability and was related to factors with higher correlation with FW incidence. Organic matter (OM), total porosity and clay content were negatively correlated with incidence, while soil density had the highest positive correlation coefficient. When cultivars were investigated, FW incidence was correlated with plantations of Silk only. There was no clear relationship between levels of management and the soil or leaves variables significantly related with principal components 1 and 2 (Fig 5a).

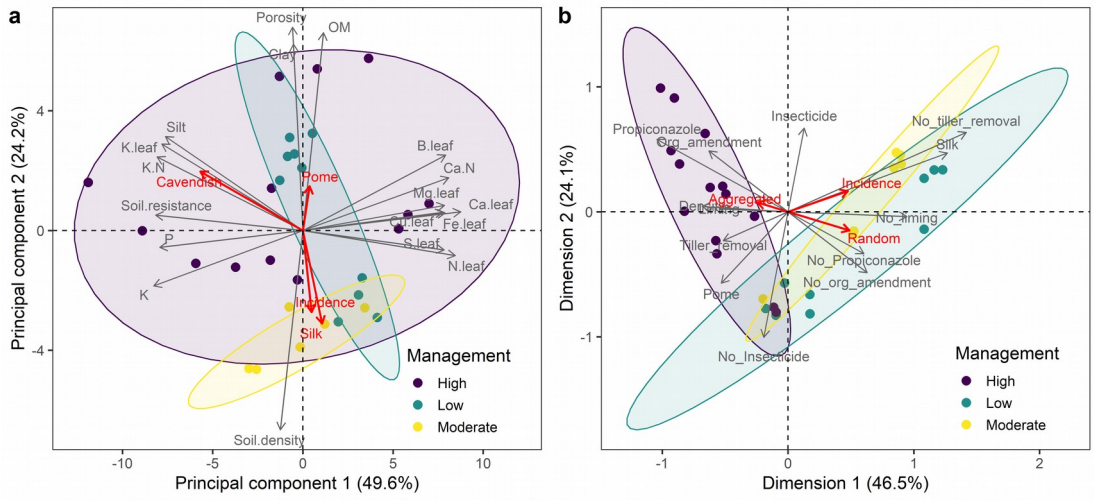



Fig 5. Principal Component Analysis (a) and Multiple Correspondence Analysis (b) from quantitative and qualitative data, respectively, from 30 fields assessed for incidence of Fusarium wilt of banana in Brazil. The coordinates of field datasets were identified by individual points and grouped according to management levels. Only significant factors ($P < 0.001$) were presented by arrows (gray). Cultivars and incidence (a); and pattern obtained by Spatial Analysis by Distances IndicEs (*SADIE*) and incidence were presented as supplementary information (red arrows). Ellipses represent the multivariate t-distribution with confidence interval of 90% for

each management level. The corresponding variance in each direction were presented in x and y axis.

Multiple correspondence analysis for categorical data of cultural practices used by farmers explained 70.6% of the total variability which was represented by eight out of 44 factors studied. The clusters formed by levels of management were well defined. In one region of the dimension 1, that explained 46.5% of the variability, fields with high levels of management were grouped together (circles) (Fig 5b). Fields with high levels of management were negatively correlated with no sucker removal, no liming, no propiconazole applications and no organic amendment, Silk cultivar and with higher FW incidences. Fields with high management levels were positively correlated with the classification of aggregation from *SADIE* while fields with moderate (squares) or low (triangles) management levels were more likely to present a random pattern of FW (Fig 5b).

## DISCUSSION

The gap of knowledge related to the spatial pattern of FW of banana has been recently raised in a review of the epidemiology of the disease (Dita et al. 2018). The present study is the most comprehensive conducted so far to estimate the intensity of the disease and its spatial pattern. In total, 95 ha, 109,280 plants were visually assessed and 7,941 diseased plants with symptoms of FW were georeferenced. Cultivar, cultural practices, density of plants, age and climate data were also collected to understand the disease intensity and spatial pattern. Additionally, soil physical and chemical properties, and foliar analysis were assessed to study the factors driving the intensity of FW. The goals of most spatial analyses are to detect spatial patterns that cannot be clearly perceived by visual inspection (Szmyt 2014). It

450 is also of interest to study the factors that affect the spatial arrangement of diseased
451 plants (Turechek and McRoberts 2013; Horne and Schneider 1995). Our study
452 employed two approaches, a spatial analysis at different scales and an investigation
453 of the processes that can affect FW epidemics.

454 The incidence of FW in Brazil was moderate (mean = 10.7%). In a study
455 conducted in two districts in Southwest Ethiopia FW incidence achieved a maximum
456 of 77% in a 100 $m^2$ field, with a mean ranging from 14.7% to 22.4% in a total of 180
457 fields (Mengesha et al. 2018). In East and Central Africa, disease incidence was
458 greater than 40% in more than 50% of the fields (Karangwa et al. 2016). The
459 moderate incidence of FW in Brazil can have socio-economic impacts because most
460 affected fields are subsistence farms. In Brazil the highest FW incidence was
461 observed in NPP and SJA regions where there is a higher proportion of susceptible
462 cultivar, Silk, planted. Cultivar resistance is effective to reduce the incidence of FW.
463 Pome and Cavendish fields were significantly less affected by the disease.

464 The aggregated pattern of FW was detected in 43% of the fields by all spatial
465 statistics and the random pattern was detected in 57% of the data sets by one or more
466 analytical methods. The quadrat-based statistics (*D* and distribution fitting) classified
467 higher number of fields in an aggregated pattern than the distance-based methods
468 (*SADIE* and point process) (Table 3). The quadrat-based statistics has a limited
469 capacity to describe the spatial pattern in the entire field and they only infer the
470 heterogeneity at scales below that the threshold at which the data were collected.
471 Quadrat-based methods do not use the spatial information of the sampling units
472 (Perry et al. 1999). Its use was important to detect the degree of heterogeneity within
473 disease clusters when few clusters were present. When quadrat-based method is used
474 in association with distance-based methods the entire process of disease spread could

be better studied. However, a clear relationship between quadrat and distance-based methods was not expected because of the intrinsic features adjusted for the different physical scales set to study spatial heterogeneity.

FW in bananas occurs in an aggregated pattern as demonstrated by the quadrat-based methods, but clusters of diseased plants (foci) can be randomly distributed in the field. Thus, at the beginning, the random localization of the inoculum determines the occurrence of a diseased banana mat. From a focus, neighboring plants are more likely to become infected originating the aggregated pattern. Consequently, aggregation is likely to be detected at lower intensity at the beginning of the epidemics and a random pattern may stand when a larger area is inspected. This was demonstrated by binary power law: fields with low incidences had weaker aggregation parameters than fields with higher incidence (Fig 4a). Soil-borne diseases caused by pathogens that can be dispersed plant-to-plant usually present this pattern (Musoli et al. 2008; Rekah et al. 1999). For coffee wilt, a disease caused by the soil-borne pathogen, *Fusarium xylarioides*, an infected plant can contaminate up to three healthy neighboring trees, in an aggregated pattern (Musoli et al. 2008). Fusarium crown and root rot of tomato caused by *F. oxysporum* f. sp. *lycopersici* (*Fol*), had an aggregated pattern, and the pathogen can spread from one plant to the closest four plants in a row by infected roots (Rekah et al. 1999). The spatial pattern of FW of banana was studied in Australia and aggregation was detected by the joint-count statistic, and isolated *Foc* TR4-infected plants were also found (Meldrum et al. 2013).

The random pattern of FW epidemics may warn about other processes affecting the dispersal of *Foc* to sites far from the main foci. In addition to plant to plant spread, weevil borer, other animals, cultural practices, wind, runoff or irrigation

water may contribute to disease dissemination (Dita et al. 2018). Aerial dispersal of *Fol* and *F. oxysporum* f. sp. *cucumerinum* has been reported (Katan et al. 1997; Scarlett et al. 2015). External sporulation was also observed for *Foc* and can act as inoculum if rain and/or wind can remove and carry the propagules to infection sites (Warman and Aitken 2018). It can be hypothesized that weevil borer (Meldrum et al. 2013), feral pigs (Biosecurity of Queensland 2016); and anthropogenic factors (Ploetz et al. 2015) could be involved in the dispersal of *Foc*.

Many biological and ecological processes can directly affect the spatial pattern of plant diseases (Campbell and Benson 1994). The study of the effect of cultivars, levels of management, plant density, age and temperature in the distribution of FW indicated that the disease has an aggregated pattern and was affected by incidence (Fig 4). The power law parameter, *A,* was significantly different between Silk and Pome cultivars. Silk is more susceptible than Pome, thus a clearer aggregated pattern was more often observed in fields planted to the latter. Based on this pattern, it is possible to infer that *Foc* can be dispersed to more distant plants and infect when epidemics occurs in highly susceptible cultivars. FW may be more restricted to neighbor plants when a less susceptible cultivar is affected. Plants with intermediate resistance levels are less affected by FW up to certain densities of inoculum (Dita et al. 2018). But, when located in areas with high densities of inoculum, such as close to diseased plants, even these plants can be infected.

Management can also affect FW epidemic (Dita et al. 2018). However, in this study regardless of management levels FW had an aggregated pattern. The high incidence of FW in fields that used intermediate levels of management did not influence the degree of aggregation. The high intensity of the disease prevented differences in aggregation levels. Many cultural practices, such as defoliation or

525 sucker removal, were not used by the farmers in fields with low management or in
526 fields with high levels of management the cultural practices were conducted with
527 more accurate technical knowledge. The practices conducted in both levels of
528 management probably contribute to reduce the spread and heterogeneity of FW in
529 fields.

530 Fields planted with high-density, old plants or in areas with low annual mean
531 temperature had higher aggregation than fields with low-density, new plantings or
532 planted in regions with high-temperature. As shown for *Fol* in tomato, it is expected
533 that *Foc* propagules do not move significant distances in soil without the presence of
534 a susceptible host (Rekah et al. 1999). High density of plants and old plantings
535 increase the probability of contact among the roots of asymptomatic and diseased
536 plants. Most likely, the younger the plantation, the greater the influence of the initial
537 inoculum on the spatial pattern. The initial inoculum can be present in the field or
538 come from outside by factors such as planting material, insects, animals, wind or
539 anthropogenic factors. Many of these can give rise to random spatial patterns. After
540 the infection by the initial inoculum, the same or many other factors can act
541 dispersing *Foc* inside the field giving rise to the observed patterns. The optimum
542 limits of temperature for the development of FW is between 22 and 31 ºC (Robinson
543 and Saúco 2011). The reduced development of FW symptoms in fields located in
544 regions with low annual mean temperature (< 22 ºC) is expected. The restrained
545 development of *Foc* reduce the inoculum production that was restricted to the
546 diseased or to immediate neighbor plants giving rise to a higher degree of
547 aggregation.

548 When all factors were analyzed using a multivariate approach, soil physical
549 properties and cultivars were the most influential factors on FW intensity. Porosity,

clay and organic matter were negatively correlated with FW incidence as previously reported (Amir and Alabouvette 1993; Deltour et al. 2017; Domínguez et al. 2001). Low incidence of FW was associated with soils with low bulk density. The more conducive soils were associated with higher sand and silt content (Deltour et al. 2017). Similarly, in Canary Islands, soils that had high clay content were more suppressive to FW on bananas (Domínguez et al. 2001). Soils with high clay content have lower bulk densities than soil with low clay and high sand contents (Jones 1983). On the other hand, no clear relationship could be observed about the variables of components 1 and 2 with the levels of management adopted by farmers. This happened probably because the cultural practices used to classify in high, moderate or low management level were not directly associated with chemical or physical soil properties.

To understand the cultural practices related to the spatial pattern of FW observed in fields, a MCA was performed. High-input fields clustered together. Although the aggregated pattern was weakly correlated with dimensions 1 and 2 of MCA, it was related with fields with high level of management practices. Removal of suckers and defoliation are some of the main practices performed in high levels of management and these practices were related with the aggregated pattern. Aggregation was also correlated with fungicide application, organic amendment, and with the Pome cultivar. On the other hand fields classified as moderate or that employed low levels of management were related with no sucker removal, no liming, Silk cultivar and with higher FW intensity and a random spatial pattern. FW intensity in a moderately susceptible cultivar was 58% lower in plots conducted with high-input management when compared with low-input techniques (Haddad et al., 2018). It was not possible to attribute any direct effect of cultural practices on the spatial

575 pattern. Long-term experiments to assess the management practices need to be
576 performed. Nevertheless, the present study supports the relation between the level of
577 technology employed and the spatial pattern of FW. In general, fields that have an
578 aggregated pattern for FW are easier to manage than those presenting a random
579 pattern of the disease.

580 There were no differences regarding the chemical properties of soils
581 associated with FW incidence. In other studies, pH (Rishbeth 1957; Deltour et al.
582 2017), electrical conductivity (EC) (Deltour et al. 2017; Domínguez et al. 2001), Ca
583 (Alvarez et al. 1981) and Zn (Jerez et al. 1983) content were negatively correlated
584 with the intensity of FW. Other chemical properties of the soil, such as organic
585 matter (OM), nitrate ($NO_3^-$), Si, K, P and B concentration were also negatively
586 correlated with FW under manipulated environments (Orr and Nelson 2018). The
587 weak association between chemical properties of soils associated with FW intensity
588 could be due to the widespread usage of lime and fertilizers. Under this situation, the
589 differences is expected to be slight. The potential association of soil physical factors
590 detected in this study may be useful to design more specific experiments to
591 investigate the effects further.

592 The intensity and spatial pattern of FW are influenced by cultivar, cultural
593 practices, soil physical properties, density and age of plantings, and annual mean
594 temperature.. These insights about the epidemiology of FW can help banana farmers
595 improve their decisions to manage the disease and reduce crop losses. Assuming the
596 general pattern of non-TR4 populations of *Foc* could be used as proxy, this large-
597 scale study is also useful to policy makers in charge of formulation of actions to
598 mitigate the consequences of the introduction of *Foc* TR4 in a region.

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# CHAPTER 2

## Spatial and temporal dynamics of Fusarium wilt of bananas and aerial dispersal of *Fusarium oxysporum* f. sp. *cubense* under field conditions

# Spatial and temporal dynamics of Fusarium wilt of bananas and aerial dispersal of *Fusarium oxysporum* f. sp. *cubense* under field conditions

**Abstract:** There is very limited information about the spatial and temporal dynamics of Fusarium wilt (FW) of bananas. The objectives of this work were to study the spatio-temporal dynamics of FW and to investigate the possibility of aerial dispersal of *Fusarium oxysporum* f. sp. *cubense* (*Foc*). Two banana fields, one planted with Prata type (Pome subgroup) and the other with Maçã (Silk subgroup) were regularly monitored for FW incidence over 24 and 12 months, respectively. In total, 3,263 banana mats were visually inspected at every two months. Symptomatic plants were georeferenced and the coordinates were used to produce distribution maps. Incidence data were used to study disease progress. Additionally, air samplers were set in the field to collect airborne propagules and to quantify the presence of *Foc* by qPCR. Incidence was low in the first (max 8.2%) but reached 63.7% at the last assessment date. The monomolecular model best fit the incidence data. Dispersion index (*D*) indicated aggregation for all plots in almost all assessments. *D* changed over time indicating higher degree of aggregation at higher incidences. Spatial Analysis by Distance Indices (*SADIE*), otherwise, indicated aggregation for 37.5% of the plots and randomness was inferred for 62.5% of the plots. Spatio-temporal association was detected for 6 of 8 plots by binary power law and significant association between successive assessments by Pearson correlation for the clustering indices. Evidence of aerial dispersal of *Foc* was confirmed and the copy number of the target gene of *Foc* was high. Precipitation and atmospheric pressure were the most consistent environmental variables associated with the weekly quantification of airborne propagules. The general pattern of the epidemics of FW

resembled that of monocyclic diseases. Aggregation was observed and the clusters of symptomatic plants were randomly distributed in most plots. The aerial dispersion of *Foc* was confirmed.

**Keywords:** Panama disease; epidemiology; aerobiology; airborne inoculum; spatial distribution.

## INTRODUCTION

Fusarium wilt (FW) of banana, caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) (E. F. Smith) W. C. Snyder and H. N. Hansen, is one of the most destructive plant diseases (Stover and Simmonds 1987) that affects banana, the world´s most important fruit (Stover 1962, Faostat 2018). The causal agent of FW is a soil-borne fungus that can infect the host through the roots. *Foc* colonizes the xylem and the rhizome, releases toxins, induces wilt and kills the plant (Stover 1962). The pseudostems of dead plants and infected roots return large quantities of *Foc* propagules to the soil. These propagules, in turn, may give rise to new infections. Epidemics of FW have been reported for decades. Lately, the banana production is at risk because of a variant of the pathogen, Tropical Race 4 (TR4), which have been dispersed from Southeast Asia to Africa and to the Middle East (García-Bastidas et al. 2014; Ordoñez et al. 2015) and now to South America (ICA, 2019). The epidemics caused by TR4 severely reduced yield in countries to where it has established (Damodaran et al. 2019; Molina et al. 2009).

In general diseases caused by soil-borne pathogens are considered to be monocyclic (Vanderplank 1963). Monocyclic diseases usually develop from small aggregates of inoculum and only one cycle of infection occurs during the crop season (Vanderplank 1963). Ploetz (2015) suggested that FW of banana is a “polycyclic”

disease because multiple cycles of infection can occur in banana plantations. The main biological characteristics of this pathosystem that support the polycyclic nature of FW are: the long crop cycle and the possibility of transmission by different ways, such as root-to-root, vectors, wind, and strong influence of cultural practices. The semi-perennial nature of the banana crops means that once established in a field it can be productive for many years (Price 1995) and, in the same way, once infected by *Foc* it could be an inoculum source for many cycles. Soil-borne pathogens such as *Phytophthora capsici* (Bowers et al. 1990), *Rhizoctonia solani* (Firman and Allen 1995), and *F. oxysporum* f. sp. *radicis-lycopersici* (*Forl*) in tomato (Rekah et al. 1999), produce multiple infections in one cycle of the host by root-to-root contact (Rekah et al. 1999). *F. oxysporum* members were also reported to be potentially dispersed by aerial processes (Rekah et al. 2000; Katan et al. 1997; Scarlett et al. 2015), but no study relating this dispersal mechanism to disease dynamics in the field was conducted. Other natural or anthropogenic ways of pathogen dispersal can affect the temporal dynamics too, and an appropriate approach to investigate this question is by comparative epidemiology analysis.

The spatial and temporal analyses of epidemics are important to understand how the disease distribution changes over time and space. It may allow us to infer about important processes affecting the pathogen dispersal in the field and the role played by different factors on the disease increase over time. A direct relationship between the initial inoculum and the spatial pattern can be seen in some cases (Ristaino and Gumpertz 2000). Anthropogenic factors have been reported as responsible for the long distance dispersal of *Foc*, mainly by informal exchange of asymptomatic seedlings, *Foc*-infested tools, infested soil adhered to shoes or tires and movement of fruit containers carrying infected parts of banana plants such as

leaf petioles (Ploetz 2015; Dita et al. 2018). In addition, *Foc*-contaminated surface water used for irrigation was responsible for the spread of FW in China, Malaysia, and the Philippines (Ploetz et al. 2015). Meldrum (2013) suggested that weevil borers (*Cosmopolites sordidus*) could be involved in the appearance of isolated banana mats with FW symptoms. Warman and Aitken (2018) observed sporodochia and hyphal growth externally on the surface of senescing leaf sheaths of infected banana plants and the aerial dispersal may be suggested. Conidia of *F. oxysporum* f. sp. *cucumerinum*, *F. oxysporum* f. sp. *basilici* (*Fob*) and *Forl* were detected in air samples and the inoculum of the last two were able to infect basil and tomato, respectively, after foliage inoculation (Scarlett et al. 2015; Rekah et al. 2000).

A proper understanding the dynamics of FW epidemics is required to plan more efficient management strategies. The lack or limited information about the spatio-temporal dynamics of FW prevents the development of more efficient strategies to manage epidemics in banana fields worldwide. The objectives of this study are to: (i) investigate the temporal dynamics of epidemics of FW in a low-input management field; (ii) understand the dynamics of the spatial pattern of FW over time; and (iii) study the aerial dispersal of *Foc.*

## MATERIAL AND METHODS

### Fields and data acquisition

One Prata (Pome subgroup) banana field with 4.4 ha established in 2012 in Teixeiras, Minas Gerais state, Brazil (42º 50’ 1.835’’ O; 20º 38’ 18.366’’ S and 749 masl) with a historical occurrence of FW was selected to study the dynamics of the disease in space in time. The field was cultivated under a low-input system, such as no sucker removal, defoliation, fertilization, or other cultural practices. Bunches

were harvested regularly during the study. Seven plots ranging from 0.3 to 1.0 ha were set in the field. The plots were delimited by the dirty roads in the area. The number of plants per plot ranged from 171 to 649. Plants were spaced approximately 3 m x 5 m, within and between rows, respectively. The plots were assessed from April 2017 to February 2019 at every two months, resulting in 12 assessments.

In addition to the plots set in the low- input system, a new field was established in an area that was not cultivated with banana. The field has 0.6 ha and was established in November 2017 with 398 plants of Maçã cultivar (Silk subgroup). The Maçã field was in the same region (42º 49' 35.371'' O; 20º 37' 50.246'' S and 687 masl) but it was located 1150 m from the Prata field. The entire Maçã field was considered as one plot. It was assessed from December 2017 to February 2019 at the same dates as previously described, resulting in eight assessments.

The incidence of FW was quantified in all banana plants of both fields at each assessment date. The plants were visually assessed for external and internal symptoms of FW. When external symptoms were observed in a plant of the mat, a small cut was made in the pseudostem to observe vascular discolorations. If external and internal symptoms were present, the mat was considered symptomatic. Zebra ribbon was used to identify the symptomatic mat which was georeferenced using a handheld GPS device (GPSMAP® 64, Garmin). When symptomatic plants were identified, fragments (5 cm of length x 5 cm of width) of pseudostems taken from 10 symptomatic plants from fields were collected to isolate the pathogen and confirm the presence of *F. oxysporum* by morphological (Leslie and Summerell 2008) and molecular methods (Lin et al. 2009; Heck et al., *in development*).

Hourly records of temperature, relative humidity and dew point were automatically taken using a HOBO data logger (Onset, Bourne, MA, USA).

Atmospheric pressure, wind velocity, radiation and precipitation data was obtained hourly from the closest climate station located at 14.4 Km from the fields and was provided by Instituto Nacional de Meteorologia (INMET) of Ministério da Agricultura, Pecuária e Abastecimento (MAPA).

**Temporal analysis**

Two data sets were constructed with the geographic coordinates: one containing the polygon and the other containing information about the location of symptomatic plants in each plot. These data sets were converted to a text file and imported into R, version 3.5.1 (R Core Team 2018). For each plot, the two data sets were used for the analyses. Polygons and diseased plants data sets were used to produce maps with the spatial distribution of symptomatic plants in each assessment.

The polygon data set was used to estimate the area, centroid and the number of plants ($n$) in each plot (i). The total number of plants in each plot was estimated using the area of the polygon and plant spacing (distance within and between rows). The diseased plant data set was used to estimate the total number and location of symptomatic plants ($x$) in plots at each assessment. The average incidence ($\bar{y}$) was calculated as $\bar{y} = x_i / n_i$, where $x$ was the number of symptomatic plants and $n$ the estimated number of total plants (including symptomatic and asymptomatic) in the $i^{th}$ plot. The incidence was calculated at each assessment time. The Monomolecular, Logistic and Gompertz models were fit to the disease incidence plotted over time by nonlinear regressions using the nlsLM function from MINPACK.LM package (Elzhov et al. 2016). The choice of the best model was performed by linear mixed-effects model (Laird and Ware 1982) using the LME function from NLME package (Pinheiro et al. 2018).

147 **Spatial analysis**

148 Two types of spatial analyses were conducted: a quadrat-based analysis, to
149 study the heterogeneity of the data at or below the physical size of the sampling unit;
150 and a distance-based analysis, to study the degree of heterogeneity between sampling
151 units in a scale above the sampling unit (Madden et al., 2007).

152 The data sets were used for quadrat-based analyses, the maps were
153 *quadratized* using the QUADRATCOUNT function of the SPATSTAT package
154 (Baddeley and Turner 2005). The quadrat size used was 2 x 2, i.e. the area occupied
155 by two plants within and two plants between rows, respectively. The number of
156 symptomatic plants in each quadrat was determined and used to perform the spatial
157 analysis. The exact number of plants inside each quadrat could not be computed
158 because the direction of rows was irregular.

159 *Dispersion index*. The index of dispersion for incidence data, $D$, sometimes
160 referred to as a relation between two variances (ratio of the observed variance and
161 theoretical variance) was used as quadrat-based method. $D < 1$ indicates a
162 underdispersed data (regular pattern); $D = 1$ indicates a random pattern and $D > 1$
163 indicates a overdispersed data (aggregated pattern). To test the null hypothesis for
164 dispersion index ($D = 1$) a $\chi^2$ test was performed. The analysis was performed using
165 the AGG_INDEX function from the EPIPHY package (Gigot 2018).

166 *Spatial Analysis by Distance IndicEs (SADIE)*. This method was used to
167 study the spatial heterogeneity in a large-scale pattern. It uses the location of the
168 sampling units (i.e. quadrats) and the number of individuals (i.e. symptomatic plants)
169 inside the unit to analyze the spatial arrangement of the diseased individuals by the
170 distance to regularity ($D_r$). In *SADIE*, the $D_r$ is achieved when all the sampling units

of a given data set (plot) have the same number of diseased individuals. The $I_a$ statistic given by *SADIE* is an overall index of aggregation obtained by the ratio between the distance moved to achieve a regular pattern for the observed data and a theoretical mean to regularity based on random permutations of the individuals among the sampling units. When $I_a < 1$, a regular pattern is inferred; if $I_a = 1$ or if $I_a > 1$, a random or an aggregated pattern is inferred, respectively. The local clustering indices $v_i$ and $v_j$, given by *SADIE*, estimate the contribution of a given unit to a patch or a gap, respectively. The index developed by Li et al. (2012) was computed by the SADIE function from EPIPHY package.

**Spatio-temporal analysis**

The relationship between the observed variance and mean of FW incidence per sampling unit across the fields and assessments was evaluated. The equation for the binary power law, $\log_{10}(s^2) = \log_{10}(A) + b \log_{10}(s^2_{bin})$, where $s^2$ is the observed variance and $s^2_{bin}$ the theoretical variance, $A$ ($\log_{10}(A)$) and $b$ are the parameters to be estimated. The null hypothesis of binary power law assumes that a random pattern for incidence data is well described by the binomial distribution. Estimates of $A = b = 1$ suggest a random distribution. If $A > 1$ ($\log_{10}(A) > 0$) and $b = 1$ then an aggregated fixed pattern across all data sets analyzed can be inferred. If $b > 1$, then the degree of overdispersion varies with $y$ and is not fixed. This value suggests higher aggregation levels with greater disease incidence values (Madden and Hughes 1995, Madden and Hughes, 1999). A t-test was performed to test the null hypothesis for a binomial distribution ($\log(A) = 0$ and $b = 1$) for individual fields and all fields.

For each plot, a temporal association analysis of the spatial pattern of FW was performedt. First the measurement of the degree of local clustering ($\chi_p$) in sampling

195 units (quadrats) was used to calculate the similarity in the cluster indices of two
196 sequential assessments. Local clustering obtained in *SADIE* analysis by the method
197 of Li et al. (2012), is a measure of the contribution for a patch or gap at that location
198 based on the original data and $D_r$. The overall association ($\chi$) was obtained by the
199 correlation coefficient of the local clustering between pairs of assessments. The t-test
200 corrected for spatial association was used to test the significance of $\chi$ (Clifford et al.
201 1989; Dutilleul et al. 1993). The analysis was performed using the SADIE and
202 MODIFIED.TTEST functions from EPIPHY and SPATIALPACK (Osorio and
203 Vallejos 2018) packages, respectively.

204 **Aerial dispersal of *Fusarium oxysporum* f. sp. *cubense***

205 *Air sample collection*. Airborne propagules were sampled using a Hirst-type
206 seven day spore trap (Burkard Scientific Ltd., Uxbridge, United Kingdom) with
207 rotating collection surface to determine the presence of *Foc* propagules in the Prata
208 and Maçã fields. The spore traps were placed in plots 5 and 8, respectively. Hirst-
209 type air sampler operates at a fixed rate of 10 $L^{-1}$ of air per minute and particles are
210 impacted on a polyester film (Melinex tape, Burkard Scientific Ltd.). The tapes were
211 manually impregnated with a thin film of liquid vaseline (Dyne, Quimibrás, RJ,
212 Brazil). The tape was 33.6 cm long by 2 cm wide, totaling an area of 67.2 $cm^2$.
213 Airborne particles were aspirated towards the surface of the tapes. The sampler was
214 placed in the middle of the plot and at a fixed height of 75 cm above the ground,
215 where air samples were taken continuously for seven days. After this period, the
216 Melinex tape was collected and changed by a new tape. Melinex tape was divided in
217 seven portions, each one corresponding to the 24-hour period of spore collection.
218 The pieces of the tape had 2 cm wide and 4.8 cm long, totaling an area of 9.6 $cm^2$.

Immediately, these individual segments were divided into smaller fragments of approximately 0.5 $cm^2$ and packed into 2 ml microtubes properly identified. Samples were stored at -20 °C to maintain the integrity and viability of the spores collected. The sampling was done weekly for 12 months, from March 2018 to March 2019 in plot 8 and for 10 weeks in plot 5, from March 2018 to June 2018.

*DNA purification.* Total DNA was extracted from the 385 air samples using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, United States of America) following the manufacturer's instructions with the following modification: Prior to DNA extraction, 0.2 g pre-sterilized glass beads (500 µm diameter) and 220 µL of 0.5% Triton X-100 were transferred into 2 mL microtubes containing the fragments kept frozen and the tissues were disrupted for 2 min using the TissueLyser II (Qiagen, Valencia, CA, USA). After the centrifugation using the "Protein Precipitation Solution" the supernatant was transferred to a clean microcentrifuge tube containing 300 µL of 2% mixed alkyl trimethyl ammonium bromide (MATAB) and 300 µL of chloroform-isoamyl alcohol (24:1 v/v) and vortexed at high speed for 20 s. Tubes were centrifuged at 14,000 rpm for 10 min and the supernatant was collected. The remainder of the DNA extraction was performed according to the manufacturer's instructions. DNA integrity was analyzed using electrophoresis on a 0.8% agarose gel in TBE (0.089M Tris base, 0.089M Boric acid, 0.002M EDTA pH 8.0) and bromophenol blue dye and the GelRED® intercalating agent were added to each well. DNA quality and quantity were determined using NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

*Airborne propagules quantification.* Foc-UFV primers (forward and reverse) were developed to amplify a conserved region of the genome of *Fusarium oxysporum* f. sp. *cubense*. The amplified fragment had 122 bp, a suitable size for a

sensitive qPCR assay (confidential data, *in development*). The primer pair was used to carry out qPCR assay, conducted in triplicate, using a Rotor Gene-Q (Qiagen). PCR reactions were performed in a volume of 10 µL, which consisted of 1 µL template DNA, 1 µL of the forward and reverse primers each (50 nM), and 5 µL of 2x QuantiFast SYBR Green PCR Master mix (Qiagen). The lightcycler conditions used were as follows: 95 °C for 5 min, followed by 35 cycles of PCR amplification at 95 °C for 10 s, and 60 °C for 30 s. Melting curves were obtained at an increasing temperature from 65 °C to 99 °C with a rise of 1 °C at every 5 s.

*Data analysis.* To monitor the airborne inoculum of *Foc* over the year the extracted DNA corresponding to seven days was mixed. The mixed DNA was analyzed by the qPCR assay described above. Copy number was expressed as $\log_n$ and presented as number of copies per $m^3$ of air. These data were related with the environmental conditions by Pearson correlation performed by COR.TEST function.

To analyze the seasonality of airborne inoculum, four seven-day periods each in the middle of each of the seasons of the year were chosen. In total, 28 days were analyzed by the qPCR assay. The daily quantification of copy number was related with the environmental conditions of the day and compared among the climatic seasons by analysis of variance and multiple comparisons test by CRD function from EXPDES package (Ferreira et al. 2018). Environmental variables were also analyzed for differences among the seasons. Data that violated normality by Shapiro-Wilk test were analyzed with the non-parametric test of Kruskall-Wallis.

## RESULTS

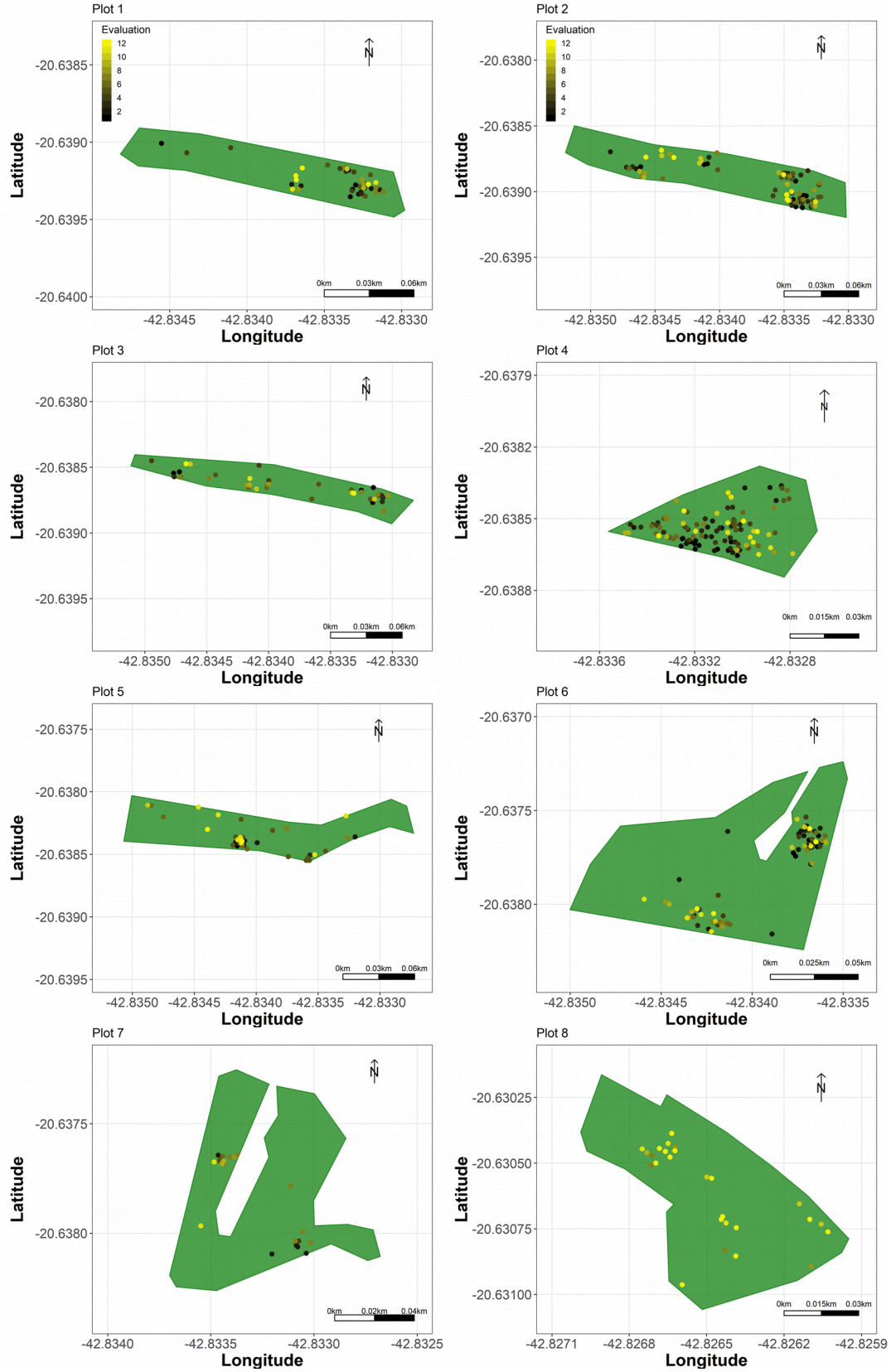
Maps of disease intensity were constructed for all eight plots (7 in the Prata field and 1 in the Maçã field) (Fig. 1). The symptomatic FW plants were identified as

268 points with different colors, from black to red, according to the assessment time.
269 Clusters and sparsely diseased plants could be observed and no spatial pattern or
270 temporal dynamics could be inferred by visual observations.

271 **Temporal analysis**

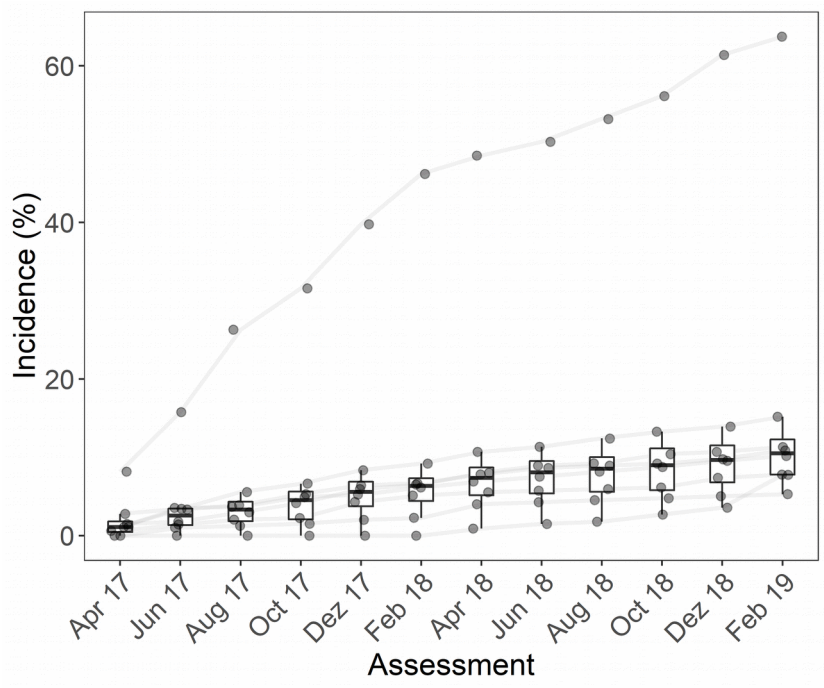
272 The incidence of FW in the first assessment date ranged from 0 to 8.19% with
273 a mean of 1.9% and median of 1.1% (Fig. 2). Six of eight plots already had
274 symptomatic FW plants before the beginning of the evaluations. In the Maçã field
275 (plot 8) the first diseased plant was observed in April 2018, 150 days after planting.
276 After six evaluations the incidence in all plots ranged from 0.9 to 48.5% with a
277 median of 7.4% and in the last assessment the FW incidence ranged from 3.9 to
278 63.7% with a median of 10.5% (Fig. 2).

279 Disease progress curves were best fitted by the monomolecular model in all
280 plots studied (Table 1; Fig. 3). The initial incidence ($y_0$) ranged from 0.001 to 0.106
281 with a median of 0.018 and the progress rate ($r$) ranged from 0.003 to 0.043, with a
282 mean and median of 0.009 and 0.004, respectively. For plot 6 the estimated $r$ value
283 was considered as an outlier, this field had the highest final incidence (63.7%) while
284 the much lower values of final incidence (< 15.2%) were recorded in other fields.


Plot 1
Evaluation
12
10
8
6
4
2
Latitude
-20.6385
-20.6390
-20.6395
-20.6400
-42.8345
-42.8340
-42.8335
-42.8330
Longitude
0km 0.03km 0.06km
Plot 2
Evaluation
12
10
8
6
4
2
Latitude
-20.6380
-20.6385
-20.6390
-20.6395
-42.8350
-42.8345
-42.8340
-42.8335
-42.8330
Longitude
0km 0.03km 0.06km
Plot 3
Latitude
-20.6380
-20.6385
-20.6390
-20.6395
-42.8350
-42.8345
-42.8340
-42.8335
-42.8330
Longitude
0km 0.03km 0.06km
Plot 4
Latitude
-20.6379
-20.6382
-20.6385
-20.6388
-42.8336
-42.8332
-42.8328
Longitude
0km 0.015km 0.03km
Plot 5
Latitude
-20.6375
-20.6380
-20.6385
-20.6390
-20.6395
-42.8350
-42.8345
-42.8340
-42.8335
-42.8330
Longitude
0km 0.03km 0.06km
Plot 6
Latitude
-20.6370
-20.6375
-20.6380
-42.8350
-42.8345
-42.8340
-42.8335
Longitude
0km 0.025km 0.05km
Plot 7
Latitude
-20.6375
-20.6380
-42.8340
-42.8335
-42.8330
-42.8325
Longitude
0km 0.02km 0.04km
Plot 8
Latitude
-20.63025
-20.63050
-20.63075
-20.63100
-42.8271
-42.8268
-42.8265
-42.8262
-42.8259
Longitude
0km 0.015km 0.03km

285

286 Fig 1. Maps for eight banana plots assessed for incidence of Fusarium wilt from
287 April 2017 to February 2019, in Teixeiras, MG, Brazil. Plots 1 to 7 were located in
288 the Prata field, and plot 8 was in the Maçã field.

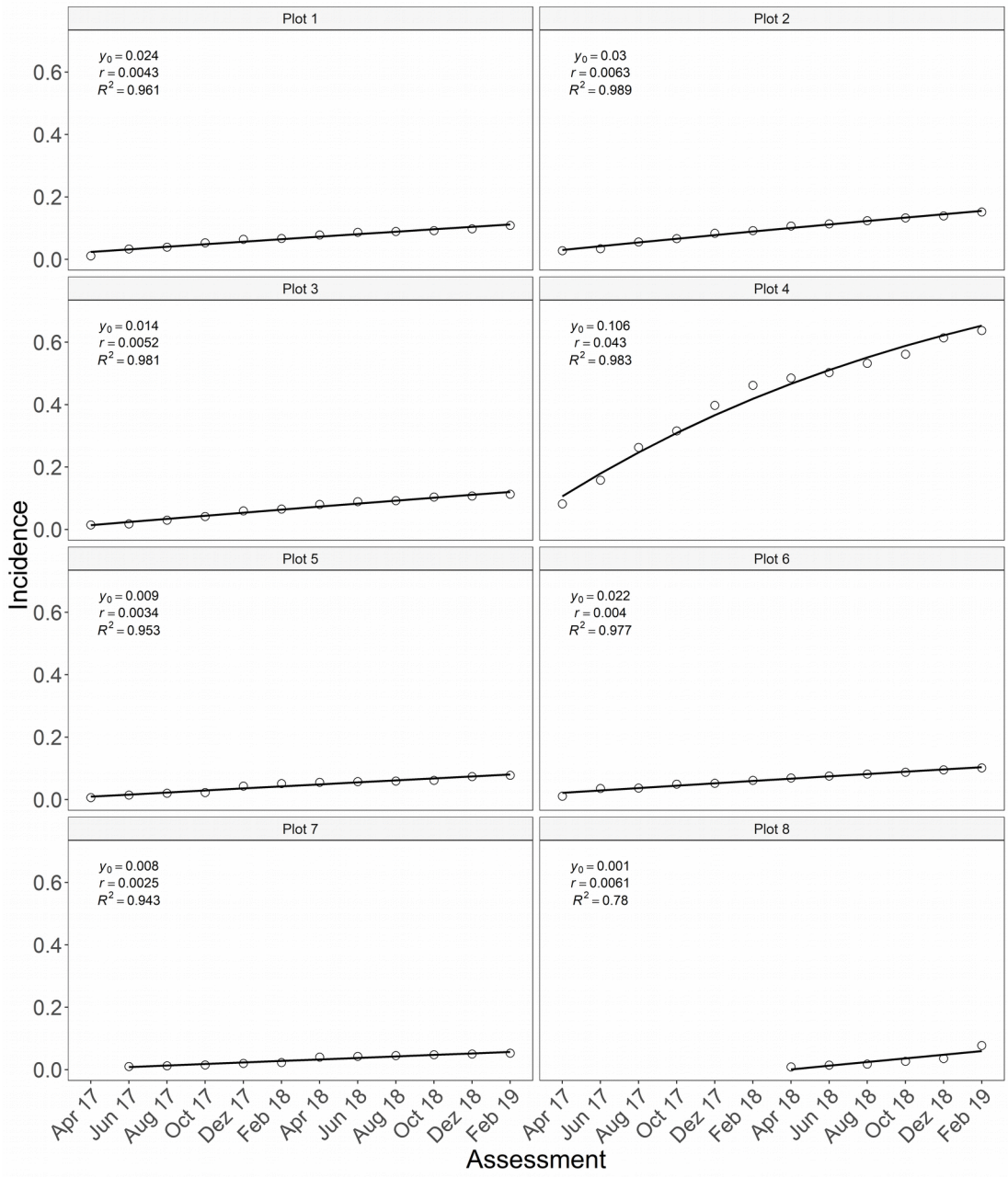
289 


290 Fig 2. Incidence of Fusarium wilt in eight banana plots assessed from April 2017 to
291 February 2018. Incidence of each plot was represented by dots and lines, median and
292 quartiles in boxplot. The curve on top of the series of boxplots refers to Fusarium
293 wilt progress in plot 4, where FW incidence was highest.

294 Table 1. Summary of statistics used to study the progress of Fusarium wilt on eight
295 banana plots located in Teixeiras, Minas Gerais state, Brazil, from April 2017 to
296 February 2019.

| Model | AIC | BIC | logLikelihood | Best adjust / *n* |
|---|---|---|---|---|
| Monomolecular | -376.84 | -335.61 | 206.42 | 8 / 8 |
| Logistic | -280.35 | -239.12 | 158.18 | 0 / 8 |
| Gompertz | -290.10 | -248.87 | 163.05 | 0 / 8 |

297 AIC: Akaike Information Criterion; BIC: Bayesian Information Criterion.



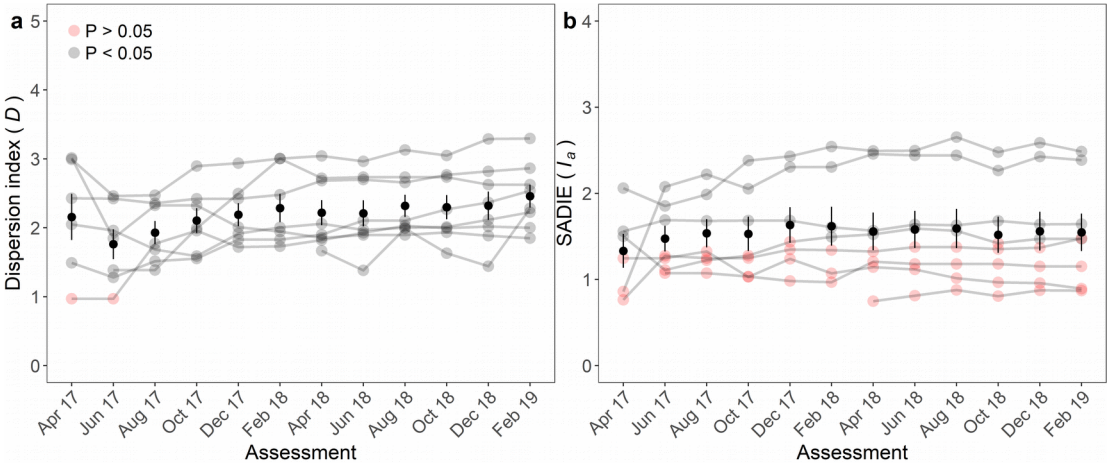

298 Fig 3. Monomolecular model adjusted to incidence data of Fusarium wilt of banana
299 from April 2017 to February 2019 in eight banana plots located in Teixeiras, Minas
300 Gerais state, Brazil. Points referred to observed incidence and integer line the
301 predicted incidence based in monomolecular model. Estimated initial incidence ($y_0$),
302 progress rate ($r$) of epidemics and the measure of the adjustment of predicted and

observed values ($R^2$) were presented. Plots 1 to 7 were located in the Prata field, and plot 8 was in the Maçã field.

**Spatial analysis**

Dispersion index ($D$) was the chosen to study the pattern of FW on banana by quadrat-based methods. In 10 of 12 assessments, banana plants with symptoms of FW were aggregated in all eight plots (Fig. 4a). In the first assessment performed in April 2017, $D$ ranged from 0.97 to 3.01 with a mean of 2.16 and in the last, February 2019, $D$ varied from 1.85 and 3.30 with a mean of 2.46. $D$ index for the eight fields increased with the FW progress. A random pattern of FW ($P = 0.61$) was observed only in plot 4 in the first two assessments ($D = 0.97$) when incidence was low ($y_{max}$ = 1.8%). The highest $D$ values was observed for plots 6 and 7 which had two defined clusters and a few sparsely diseased plants.

Aggregation of banana plants was confirmed by *SADIE* ($P < 0.05$), a distance-based method, in plots 1, 2 and 6, which correspond to 37.5% of the plots analyzed. Plots 3, 5, 7 and 8 had a random pattern ($P < 0.05$) in all assessments. Plots 4 had an inconsistent pattern along the study period, varying between random and aggregated. In the first assessment, April 2017, $I_a$ ranged from 0.76 to 2.06 with a mean of 1.33 and in the last, February 2019, $I_a$ varied from 0.87 to 2.48 with a mean of 1.55. These results revealed that the index of aggregation of *SADIE* remained relatively stable over time (Fig 4b).



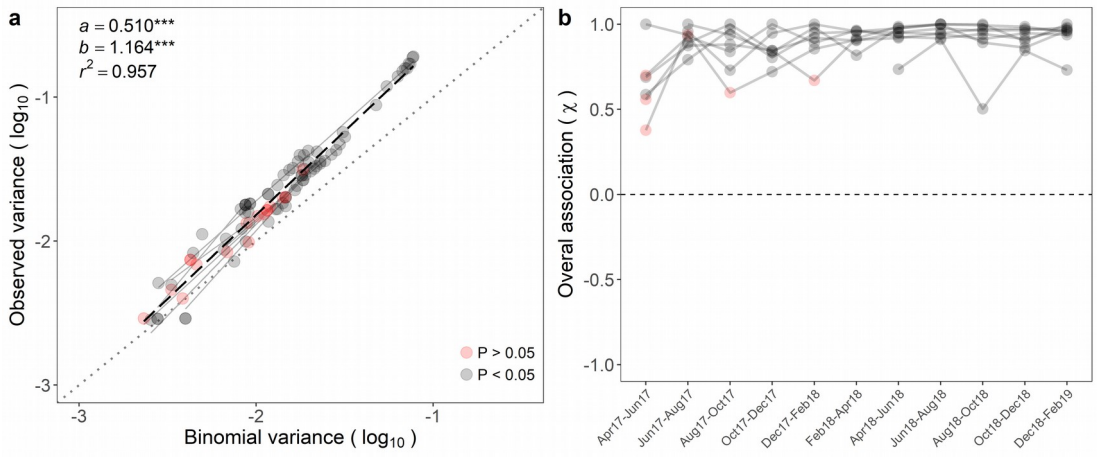

323 Fig 4. Dispersion index (*D*) (a) and aggregation index from Spatial Analysis by
324 Distance IndicEs (*SADIE*; $I_a$) (b) for incidence of Fusarium wilt of banana from April
325 2017 to February 2019 in 8 plots located in Teixeiras, Minas Gerais, Brazil. Red and
326 gray points were presented when the null hypothesis of randomness was not rejected
327 ($P > 0.05$) or rejected ($P < 0.05$), respectively. Error bars represent standard error and
328 black point the means.

329 **Spatio-temporal analysis**

330 The relationship between the logarithm of the observed variance and the
331 logarithm of the mean for the eight plots in 12 bimonthly assessments was well
332 described by the binary power law ($R^2 = 0.96$) (Fig 5a). Parameters from power law,
333 $\log_{10}(A)$ (0.510 ± 0.051) and $b$ (1.164 ± 0.026), were significantly ($P < 0.001$)
334 different from 0 and 1, respectively, when all plots were jointly analyzed. As $\log_{10}(A)$
335 was higher than 0 and $b$ was higher than 1, in general FW exhibited an aggregated
336 pattern and the degree of aggregation increased with disease incidence. When the

337 plots were analyzed individually over time, the correlation coefficient of
338 determination ($R^2$) ranged from 0.957 to 0.992, $\log_{10}(A)$ was between -0.03 to 1.25
339 and $b$ varied between 0.90 to 1.45. Six of eight plots (75%) exhibited an aggregated
340 pattern with $\log_{10}(A)$ and $b$ significantly higher than 0 and 1, respectively (Fig. 5a).
341 The null hypothesis of randomness was not rejected by the binary power law in plot
342 5 ($\log_{10}(A) = -0.031 \pm 0.09$; $b = 0.903 \pm 0.04$; $P = 0.418$) and plot 8 ($\log_{10}(A) = 0.382$
343 $\pm 0.260$; $b = 1.124 \pm 0.120$; $P = 0.096$).

344 Spatial associations were detected between the pairs of successive
345 assessments in local clustering ($P < 0.05$) (Fig. 5b). In the beginning of FW
346 epidemics, there was no association between the first (April 2017) and second (June
347 2017) observations in three (plots 1, 5 and 6) of five plots ($P = 0.334$; 0.189 and
348 0.741, respectively). No association was observed for plot 3 between June and
349 August ($P = 0.058$) and between August and October 2017 ($P = 0.186$), and for plot
350 5 between December 2017 and February 2018 ($P = 0.077$; Fig. 5b). After the fifth
351 comparison, December 2017 and February 2018, all comparisons of the local
352 clustering were significantly associated for all plots.




353 Fig 5. Binary power law (a) and overall association (b) of successive assessments
354 (pairs) of clustering indices for incidence of Fusarium wilt of banana from April
355 2017 to February 2019 in 8 plots located in Teixeiras, Minas Gerais, Brazil. Red and
356 gray points were presented when the null hypothesis of randomness was not rejected
357 ($P > 0.05$) or rejected ($P < 0.05$), respectively.

358 To study the spatial associations between observations more spread further
359 apart in time, comparisons between the first and middle or latest observations of the
360 epidemic, and between the middle and last assessments were performed (Table 2).
361 Plots 4 and 7 were positively associated ($R > 0.543$; $P < 0.046$) for the three
362 assessment times. Plot 2 exhibited significant association between the first and the
363 middle ($P = 0.03$) and between the middle and the last ($P < 0.001$) assessments of the
364 epidemic. No spatial association was observed for the other plots between the first
365 and middle assessments (Table 2). Plots 1, 3, 5, 6 and 8 were spatially associated
366 when comparing between the middle and latest observations (Table 2).

Table 2. Overall association of the spatial pattern of Fusarium wilt of banana epidemics between three assessment times (first, middle and last assessment) for eight fields.

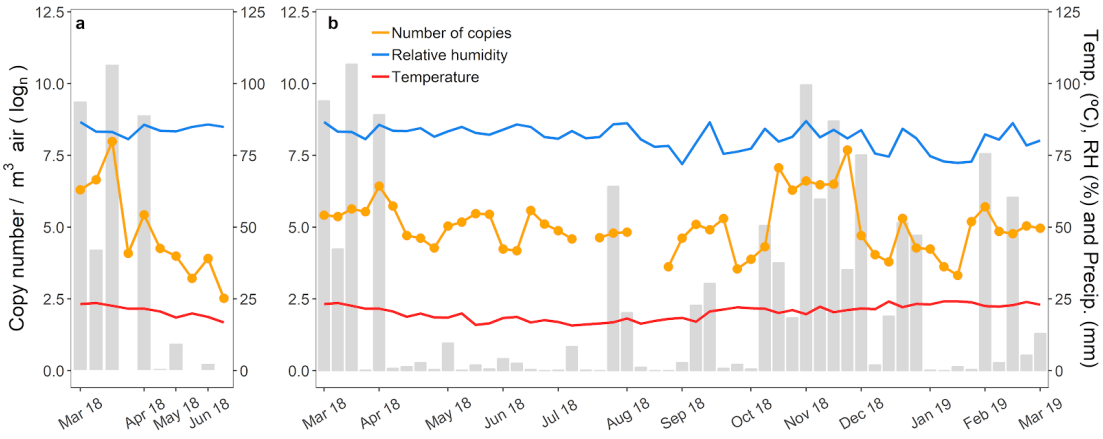
| Plot | Assessment 1 | Assessment 2 | Overall association (*P*-value) [a] |
|---|---|---|---|
| 1 | April 2017 | February 2018 | 0.367 (0.264) |
| | February 2018 | February 2019 | **0.864 (0.004)** |
| | April 2017 | February 2019 | 0.253 (0.423) |
| 2 | April 2017 | February 2018 | **0.611 (0.030)** |
| | February 2018 | February 2019 | **0.781 (<0.001)** |
| | April 2017 | February 2019 | 0.573 (0.019) |
| 3 | April 2017 | February 2018 | 0.441 (0.106) |
| | February 2018 | February 2019 | **0.735 (<0.001)** |
| | April 2017 | February 2019 | 0.303 (0.227) |
| 4 | June 2017 | February 2018 | **0.563 (0.046)** |
| | February 2018 | February 2019 | **0.937 (<0.001)** |
| | June 2017 | February 2019 | **0.543 (0.045)** |
| 5 | April 2017 | February 2018 | 0.499 (0.237) |
| | February 2018 | February 2019 | **0.853 (<0.001)** |
| | April 2017 | February 2019 | 0.433 (0.253) |
| 6 | April 2017 | February 2018 | 0.577 (0.106) |
| | February 2018 | February 2019 | **0.814 (<0.001)** |
| | April 2017 | February 2019 | 0.463 (0.113) |
| 7 | June 2017 | February 2018 | **0.880 (<0.01)** |
| | February 2018 | December 2018 | **0.754 (0.001)** |
| | June 2017 | December 2018 | **0.663 (0.004)** |
| 8 | April 2018 | October 2018 | 0.206 (0.262) |
| | October 2018 | February 2019 | **0.698 (<0.001)** |
| | April 2018 | February 2019 | 0.202 (0.243) |

[a] Overall association based on Pearson correlation was calculated using the clustering index from Spatial Analysis by Distances IndicEs (*SADIE*). *P*-value was obtained from a modified t-test based on Clifford et al. (1989) and Dutilleul (1993). Significant correlations are shown in bold ($P < 0.05$).

374 **Aerial dispersal of *Fusarium oxysporum* f. sp. *cubense***

375 Air sampling collection was successfully performed for 10 weeks in Prata
376 field, from March to June 2018, and 50 weeks in Maçã field, from March 2018 to
377 March 2019. The copy number detected by qPCR varied over the weeks (Fig 6). In
378 Prata field the copy number ranged from 12.5 to 2944.4 per $m^3$ of air, with a median
379 of 65.3 over 10 weeks. In Maçã field copy number ranged from 27.8 to 2177.8, with
380 a median of 139.6 per $m^3$ of air over 50 weeks. No significant difference could be
381 detected between the copy number ($log_n$) of both fields considering the same period.

382 Relationship between environmental conditions and density of copies in air
383 samples was verified using Pearson correlation analysis. Temperature (R = 0.843, *P*
384 = 0.002) and dew point (R = 0.841, *P* = 0.002) were significantly correlated with
385 copy number in Prata field. Relative humidity (RH) was significantly correlated (R =
386 0.391, *P* = 0.005) with copy number in Maçã field. Atmospheric pressure (hPa) (R =
387 -0.820 and -0.283) and precipitation (R = 0.86 and 0.472) were correlated (*P* <
388 0.002) with the copy number in both fields. There were no significant correlations
389 between wind velocity and solar radiation with the copy number in any of the
390 evaluated fields.




391 Fig 6. Average copy number ($log_n$) per $m^3$ of air (orange line) of *Fusarium*
392 *oxysporum* f. sp. *cubense* at weekly mixed samples from March 2018 to March 2019
393 in Prata (a) and Maçã (b) fields. Average of temperature (Temp.) and relative
394 humidity (RH) were presented as red and blue lines, respectively, and cumulative
395 precipitation (Precip.) as gray bars.

396 Daily quantification of copy number ($log_n$) did not differ significantly among
397 the seasons (Table 3). Temperature, dew point and atmospheric pressure were the
398 climatic factors that differed among seasons ($P < 0.001$). Temperature and dew point
399 were higher in summer and spring than in winter and autumn; while atmospheric
400 pressure was higher in autumn and winter than in summer and spring (Table 3).
401 Relative humidity, wind velocity, radiation and precipitation did not differ among
402 seasons (Table 3). No correlation among the environmental data from the different
403 seasons and copy numbers was detected (data not shown).

404 Table 3. Seasonality of copy number of *Fusarium oxysporum* f. sp. *cubense* airborne propagules and climatic conditions.

| Season[a] | Copy number ($log_n$)$^{ns}$ | Temperature (ºC) | Relative humidity (%)$^{ns}$ | Dew point (º C) | Atmospheric pressure (hPa) | Wind velocity ($m.s^{-1}$)$^{ns}$ | Radiation ($kJ.m^{-2}$)$^{ns}$ | Precipitation ($mm.day^{-1}$)$^{ns}$ |
|---|---|---|---|---|---|---|---|---|
| Summer | 5.35 | 23.2 a | 88.2 | 20.3 a | 934.3 b | 0.66 | 617.3 | 10.8 |
| Autumn | 5.74 | 16.0 b | 82.9 | 12.6 c | 939.3 a | 0.50 | 498.1 | 0.3 |
| Winter | 4.89 | 17.0 b | 92.6 | 15.3 b | 938.9 a | 0.52 | 392.0 | 9.2 |
| Spring | 5.49 | 21.5 a | 92.4 | 19.5 a | 935.1 b | 0.94 | 696.6 | 2.6 |
| CV (%) | 21.6 | 8.6 | 7.7 | 9.5 | 0.26 | 52.7 | 42.7 | 127.3 |

405 [a] Data were obtained from daily quantification of seven days on each season.

## DISCUSSION

One of the biggest constraints to develop efficient management strategies to FW on banana is the lack of information about the basic epidemiological processes of the disease: the spatial and the temporal dynamics. Studies involving the spatial and temporal dynamics of FW on banana fields are absent and gaps in epidemiology are constantly emphasized in reviews (Dita et al. 2018; Ghag et al. 2015; Ploetz 2015). To fill some of these gaps bimonthly assessments of FW incidence with georeferenced data were conducted during two years.

Visual maps of diseased plants did not allow to conclude about the dynamics of FW over time and space. Plots 1 to 7 were cultivated with 'Prata' type banana since 2012, and had a historical occurrence of FW while plot 8 was a new field cultivated with 'Maçã', a highly susceptible cultivar. Overall the FW incidence was low in almost all plots (median = 10.5%); much lower than values reported in Indonesia and Africa (Hermanto et al. 2011; Karangwa et al. 2016; Mengesha et al. 2018). Plot 5 was the exception where the epidemic developed fast and reached up 63.7% of incidence on February 2019. Similar values of incidence were reported in Ethiopia (77%) (Mengesha et al. 2018) and Tanzania (63.6%) (Karangwa et al. 2016) in plantations at final production cycles.

In general diseases caused by soil-borne pathogens are considered monocyclic (Vanderplank 1963). Ploetz (2015) suggested that FW of banana is a "polycyclic" disease because multiple cycles of infection can occur in banana

427 plantations affected by *Foc*. However, the results showed that the monomolecular
428 was the best fitted model to the data over two years of assessments in a low-input
429 farm. Many characteristics of the pathosystem suggest otherwise. The semi-perennial
430 nature of the banana plant means that once infected by *Foc* it could be a inoculum
431 source for many years to neighboring plants. The need of intensive management to
432 achieve high productivity and other mechanisms of dispersal could affect the
433 dynamics of FW, as reported for other diseases caused by *F. oxysporum* (Rekah et al.
434 1999; Scarlett et al. 2014, 2015). Similar argument was suggested for *Foc* (Meldrum
435 et al. 2013). In the current study, there were limited cultural practices conducted in
436 the field. Thus, *Foc* dispersal could be related to undocumented factors, such as
437 natural occurrence of *Foc* in the soil, asymptomatic seedlings, root-to-root, vectors,
438 floods or wind.

439 Spatial pattern analysis can shed light on the mechanisms involved in the
440 spread of FW and on pathogen dispersal. Based on the dispersion index, there was
441 evidence for an aggregated pattern of FW epidemics. Regardless of the location of
442 the sampling unit, there was higher heterogeneity in the number of symptomatic
443 plants inside the sampling units compared with FW incidence. In addition, the degree
444 of aggregation changed over time, lowest *D* values were observed at the beginning of
445 FW epidemics and increased as incidence increased. After the initial foci were
446 formed the asymptomatic plants located near symptomatic ones were the first to be
447 affected by FW. Similar results were reported with the use of quadrat-based methods

448 for epidemics caused by soil-borne pathogens (Musoli et al. 2008; Rekah et al.
449 1999).

450 Distance-based methods detected a different pattern and divergences were
451 observed between $D$ and $I_a$. Aggregation was consistently observed in 37.5% (3/8) of
452 the plots, while randomness could be detected in 62.5% (5/8) by *SADIE*. The method
453 quantified the degree of contribution of each sampling unit towards the overall
454 degree of clustering achieved by $D_r$ (Perry et al. 1999). On the process to reach
455 regularity ($D_r$), one cluster of diseased plants (donors) was considered one patch and
456 regions without diseased plant as gaps (receptors) (Perry et al. 1999). If the clusters
457 were sparsely dispersed, the number of clusters or the degree of aggregation in
458 clusters were low, what could be seen in plots 3, 5, 7 and 8, the probability of not
459 rejecting the null hypothesis of randomness was high. From the first to last
460 assessments a small variation in the degree of aggregation by *SADIE* was observed.
461 Probably, in this case, the initial inoculum reached the plots by unobserved
462 mechanisms, as symptomatic seedlings, vectors and wind, which gave rise to a
463 random pattern. Once established in the field, plant-to-plant transmission may have
464 contributed to the increase of the degree of aggregation of FW.

465 Strong evidence of aggregation was indicated by the binary power law over
466 time. Power law was used as a spatio-temporal analysis and considered all
467 assessments for each plot. The degree of aggregation was affected by the level of
468 incidence itself and its rate of increase over time. In only two plots (5 and 8) the null

hypothesis of randomness was not rejected. This result did not agree with *D* statistics, that is also a ratio between variances, but *D* considers only one assessment at a time to test for spatial pattern while binary power law considers all assessments through time (Laranjeira et al. 2000). Plot 8 resulted in the lowest values for *D* and for binary power law. Temporal associations of the clustering indices suggest correlations between the successive pairs of assessments. Only in the beginning of the epidemic, when the incidence was low, there were some fields with no spatial association (Fig. 5b, Table 2). This fact was expected because the new infections in different sampling units contribute to the lack of association over time. However, association between the clustering indices of middle and last assessment of the epidemic was observed. Turechek and Madden (1999) reported that spatial association was only detected when the clusters of disease expanded beyond the borders of the sampling units. As demonstrated by *D* and power law, aggregation was higher when incidence achieved higher values until the maximum observed (63%).

The initial inoculum seems to drive the epidemics of monocyclic diseases (Jeger 1987). Understanding how the inoculum arrives in the field and the mechanisms involved in *Foc* dispersal are key to propose efficient management strategies to avoid FW epidemics or, at least, reduce crop losses. As *SADIE* detected a consistent random pattern in some plots it is an indicative that the inoculum could have arrived from outside or have been dispersed not by root-to-root contact or

490 neighboring plants. Asymptomatic seedlings, muddy boots, *Foc*-infested tools, soil
491 with *Foc* propagules adhered to machinery tires, mammals, insects, and wind are
492 some of the factors suggested that can introduce *Foc* in new areas (Biosecurity of
493 Queensland 2016; Dita et al. 2018; Meldrum et al. 2013; Ploetz et al. 2015).

494 In the present study *Foc* propagules were detected over one year of air
495 sampling. Monitoring was made weekly and spores were detected on 100% of the
496 samples analyzed at relatively high rates (median of 65 to 140 copies per $m^3$ of air).
497 The copy number did not reflect the exact number of propagules because macro- and
498 microconidia of *Fusarium* species had different number of cells. *F. oxysporum* f. sp.
499 *cucumerinum* was detected in air samples of a greenhouse at rates that ranged from 1
500 to 140 copies per $m^3$ of air (Scarlett et al. 2015). Both macro- and microconidia were
501 detected in air samples but failed to establish infection on stem wounds of cucumber
502 (Scarlett et al. 2015). These high rates observed in the air samples could be addressed
503 by the proximity to a source of inoculum in the field. In Prata field the closest
504 diseased mat was located approximately 5 m from the air sampler in Northwest
505 direction. At longer distances diseased mats were present in all directions. In Maçã
506 field no symptomatic plants were present inside the plot at the beginning of the
507 monitoring. Diseased mats were only observed at the potential source of inoculum,
508 an abandoned banana plantation, located approximately 80 m from the sampler in the
509 South direction. No significant correlation could be detected between density of
510 propagules in the air and the incidence of FW in fields.

511 Evidence for aerial dispersal of *Foc* propagules has accumulated over the
512 years, but its epidemiological role remains elusive. *Fol* was observed sporulating on
513 stems of cherry tomatoes growing in greenhouses and airborne propagules were
514 trapped on selective medium (Katan et al. 1997). *Forl* and *Fob* were also detected
515 sporulating in stems of tomato and basil, respectively, and airborne propagules were
516 trapped by selective medium (Rekah et al. 2000). *Foc* SR4 was also able to grown in
517 outer surface of pseudostem of banana infected plant on pathogenicity tests in
518 greenhouse (Fig S1). Morphological (Leslie and Summerell 2008) and molecular
519 (Lin et al. 2009) methods confirmed the presence of *Foc* externally. However, in the
520 field, external sporulation was not observed during the monitoring study. Warman
521 and Aitken (2018) observed an isolate of *Foc* SR4 genetically transformed with
522 green fluorescent protein (GFP) in the outer surface of asymptomatic plants and
523 sporodochia and hyphal growth on the outer surface of senescing leaf sheaths.
524 Propagules produced in hyphae and sporodochia grown in the outer surface of plants
525 can be released and carried long-distance by winds. However, to date, the capacity of
526 aerially-dispersed propagules to infect banana plants has not been demonstrated.
527 Environmental conditions may influence the production, release, survival and
528 deposition of these spores loaded by wind. On weeks with higher cumulative rainfall
529 higher number of propagules were detected in air samples. Weeks with lower
530 atmospheric pressure had higher number of airborne propagules. Average
531 temperature, relative humidity and dew point were also related with the density of

propagules, but the effects were not consistent between the two fields analyzed. Changes in relative humidity could have some relation with changes in airborne propagules of *F. oxysporum* f. sp. *cucumerinum*, but no conclusion was reached (Scarlett et al. 2015). High humidity was also suggested as having some relation with *Fol* sporulation in external surface of tomato stems (Katan et al. 1997). The highest spore number of *Fusarium circinatum* in air samples was counted in autumn, suggesting a seasonal fluctuation (Schweigkofler et al. 2004). Airborne propagules of *Foc* did not show a seasonal fluctuation and only temperature, dew point and atmospheric pressure differed among the seasons.

The dynamics of FW of banana was partially clarified in the present study. However, it is important to point out that temporal, spatial and spatio-temporal characteristics of FW epidemics on a banana subsistence farm may not be the same as FW epidemics in other fields. The management system, cultivars, type of soil and climatic conditions are key factors to the development of FW epidemics and can vary from field to field (Dita et al. 2018). In addition to plant-to-plant, transmission of FW can involve other processes. Evidences of aerial dispersion of *Foc* propagules was highlighted here. The airborne propagules identified in this study are probably of *Foc* SR4 or race 1 (data not shown). Using this as a proxy, *Foc* TR4 may also be dispersed by aerial mechanisms, therefore, it can put in alert the banana production system worldwide. Additionally, this could explain the fast spread of the FW epidemics in Asia and Middle East caused by *Foc* TR4 and the transcontinental jump

553 in recent years (Chittarath et al. 2018; Damodaran et al. 2019; Dita et al. 2018;
554 García-Bastidas et al. 2014; Hung et al. 2018; ICA, 2019; Maymon et al. 2018;
555 Ordoñez et al. 2015; Ploetz et al. 2015; Zheng et al. 2018). This work can be used to
556 direct similar dynamic studies in other regions to advance the understanding of FW
557 epidemics and direct aerobiology studies of *Foc* to give rise on efficient management
558 strategies in future years.

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## SUPPLEMENTARY MATERIAL

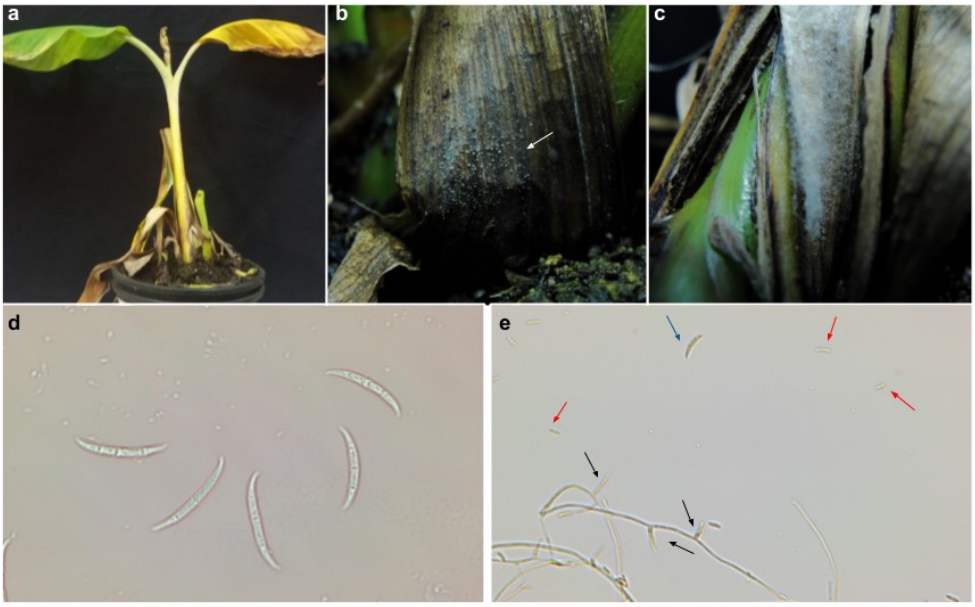



Fig S1. Banana cv. Maçã with symptoms of Fusarium wilt grown in 1 L pots under greenhouse conditions (a). Roots were artificially inoculated with a *Fusarium oxysporum* f. sp. *cubense* isolate (*Foc*UFV-580) by root dipping. Sporodochia (white punctuations) in the outer surface of banana pseudostem (b). Hyphal growing in outer surface of disrupted pseudostem (c). Macroconidia recovered from sporodochia (white arrow) produced externally (d). Macro- (black arrow), microconidia (red arrow) and phialides (black arrow) recovered from hyphal growing externally in the pseudostem (e).

# CHAPTER 3

## Weevil borers and the spatio-temporal dynamics of Fusarium wilt of banana

# Weevil borers and the spatio-temporal dynamics of Fusarium wilt of banana

**Abstract:** Dispersal of propagules of a pathogen has remarkable effects on the development of epidemics. Previous studies have suggested the role of banana insect pests on the development of Fusarium wilt (FW) epidemics. Here we provide complementary evidence for the involvement of two insect pests of banana, *Cosmopolites sordidus* L. (WB) and *Metamasius hemipterus* L. (FWB), in the dispersal of *Fusarium oxysporum* f. sp. *cubense* (*Foc*) using two approaches: a comparative epidemiology study under field conditions and an association analysis between pests and *Foc*. Two banana fields with historical records of FW epidemics were used, one managed with *Beauveria bassiana* to reduce the population of WB, and the other was left without management. The number of WB and FWB was monitored during two years and the FW incidence was also quantified. The population of WB and FW incidence (6.7%) in the field managed with *B. bassiana* were lower than in the field left unmanaged (13.0%). The monomolecular model best fit the FW incidence data and, as expected, the average estimated disease progress rate was lower in the field managed with the entomopathogenic fungus (r = 0.0024) compared to the unmanaged field (r = 0.0056). Aggregation of FW was higher in field with WB management. The association analysis revealed a greater number (7,933) of colony forming units (CFU) of *Fusarium* spp. associated with WB (n = 115 insects) than with FWB (1,414 CFU; n = 102 insects). WB affects the spatial and temporal dynamics of FW epidemics under field conditions. *Fusarium* spp. was associated with both pests but *Foc* was not detected.

**Keywords:** *Fusarium oxysporum* f. sp. *cubense*, Panama disease, spatio-temporal, banana weevil, West Indian sugarcane weevil

## INTRODUCTION

Understanding the ways a pathogen is dispersed is one of the most important tasks in epidemiology of plant diseases. Without pathogen dispersal no epidemics occurs. For Fusarium wilt (FW) of bananas, caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) (E. F. Smith) Snyder and Hansen, there is limited information about the mechanism of pathogen dispersal available. It is suggested that *Foc* can be dispersed mainly by human influence, such as exchange of asymptomatic propagation material, cultural practices performed with infested tools, and soil particles adhered to boots, machinery and tires of vehicles (Dita et al. 2018; Ploetz et al. 2015). Good management practices, sterilization of materials and appropriate training of plantation workers can greatly reduce dispersal and the likelihood of introduction of the pathogen in clean areas.

The influence of natural dispersal agents such as wind, rivers, animals, including mammals and insects are more difficult to detect and to control, but they certainly influence the development of epidemics. Some reviews highlighted the potential of these dispersal processes of *Foc* at short or long distances (Dita et al. 2018; Ghag et al. 2015; Ploetz 2015; Ploetz et al. 2015). Surface water and rivers infested with *Foc* propagules were putatively associated with the rapid expansion of FW epidemics in China, Malaysia and the Philippines (Ploetz et al. 2015; Xu et al. 2003). *F. oxysporum* species does not seem to move long distances in soil. Inoculation normally occurs when root growth contacts the inoculum distributed in the soil or by root-to-root contact (Rekah et al. 1999). The presence of sporodochia and hyphal growth externally in plant tissues suggests that aerial dispersal of *Foc* is possible (Warman and Aitken 2018). The free circulation of animals such as feral

pigs in banana fields was cited as a potential mechanism of dispersal of *Foc* (Biosecurity of Queensland 2016). Smaller animals such as insects, banana weevil borer (*Cosmopolites sordidus* L., Coleoptera: Curculionidae), referred here as weevil borer (WB), in particular, probably play a role in the inoculation of isolated banana plants in the field (Meldrum et al. 2013). The WB can occur in large numbers in banana fields and can contribute to spread the disease, but no conclusive results have been reached so far.

Any animal or material that can carry soil particles in banana fields is a potential dispersal agent of *Foc*. Weevil borer, is a main pest in banana fields (Gold et al. 2001). West Indian sugarcane weevil (*Metamasius hemipterus* L., Coleoptera: Curculionidae), referred as false weevil borer (FWB), is an important pest in sugarcane, but can also cause damage in banana under high populations and is commonly observed in many banana fields (Fancelli et al. 2012). Curculionidae members acting as vectors of plant diseases are commonly reported in some crops. *M. hemipterus* and *Rhynchophorus palmarum* (Coleoptera: Curculionidae) were suspected to be vectors of the nematode *Bursaphelenchus cocophilus* (=*Rhadinaphelenchus cocophilus*), causal agent of red ring disease in oil palm (Mora et al. 1994; Hagley 1963). *R. palmarum* was also reported as dispersal agent of bud rot disease (*Phytophthora palmivora*) in oil palm (Plata-Rueda et al. 2016) and stem bleeding (*Thielaviopsis paradoxa*) in coconut palm (Carvalho et al. 2011). However, since the study carried out by Meldrum et al. (2013) the potential of banana weevil to disperse *Foc* remains unanswered. Nevertheless, these answers may be useful to the development of preventive measures against quarantine pathogens, as *Foc* Tropical Race 4 (*Foc* TR4), and effective management strategies to control the expansion of FW foci within the field. The objective of this study was to understand the

relationship of two potential vectors of *Foc*, *C. sordidus* and *M. hemipterus*, using two approaches: (i) a comparative epidemiology study under field conditions and (ii) an association study between the potential vectors and *Foc.*

## MATERIAL AND METHODS

### Comparative epidemiology

*Field experiment*

Two banana plots with history of epidemics of FW were evaluated in Teixeiras, Minas Gerais state, Brazil, from March 2016 to April 2017. These plots were cultivated with a Prata type banana (Pome subgroup) and managed in a low-input technology system. In one plot (20° 38' 21.444" S; 42° 50' 1.752" W and 772 masl) of 1.03 ha WB was managed while the second plot (20° 38' 19.5" S; 42° 50' 2.544" W and 759 masl) of 1.2 ha remained without any control practice for WB. The management of WB was conducted at every three months. Carbofuran (Furadan 50 G®, FMC Corporation) was applied manually at 3 g of commercial product per trap once at the beginning of the experiment. Additionally, five grams of Ballvéria WP® ($10^9$ CFU of *Beauveria bassiana* per gram of commercial product) was applied in each trap. Applications of *B. bassiana* were made at every 3 months in 30 to 40 pseudostem traps in the plot with control of WB. Traps were constructed by cross-cutting pseudostems of harvested plants at approximately 40 cm from the soil line. The biological insecticide was applied on the flat, cut surface, of one half of the trap, and the other half was placed back on top of the treated half.

*Weevil borer monitoring*

A second type of pseudostems traps were used to monitor the WB population. Pseudostems of harvested plants were cross cut in sections of 30 cm and subsequently lengthwise in half such as to produce two hemi-cylindrical traps. The sections were disposed with cut side down (in contact with soil). Fifteen pseudostem traps per plot were made and placed approximately 15 cm apart from the banana mat. The traps were randomly distributed in the plots and maintained in the same place throughout the experiment. Monitoring was performed from May 2017 to February 2019. Traps were evaluated by counting the number of WB and FWB at every 14 days and replaced by new ones.

*Fusarium wilt assessments*

Banana plants were visually assessed for external and internal symptoms of FW. When external symptoms were observed, a small cut was made in the pseudostem to inspect for internal symptoms. If external and internal symptoms were present, the mat was considered symptomatic and was georeferenced using a handheld GPS device (GPSMAP® 64, Garmin). The FW symptomatic banana mat was identified with a zebra ribbon to avoid double mark in future assessments. GPS files with geographic location of symptomatic plants and polygons were extracted and converted to text files and imported in R Statistical Software version 3.5.1 (R Core Team 2018). Fragments (5 cm of length x 5 cm of width) of pseudostems taken from symptomatic plants from each field were collected to isolate the pathogen and to confirm the presence of *Foc* by morphological (Leslie and Summerell 2006) and molecular methods (Heck et al. *in development*).

*Data analysis*

*Insect population*. The paired t-test was used to analyze differences between the average number of WB and FWB in each assessment date. T-test was also used to analyze differences in the average number of WB and FWB assessed between the managed and unmanaged plots.

*Temporal analysis*. The area, centroid and the total number of plants in each plot were estimated using the polygon data set and the plant spacing information (distance within and between rows). The symptomatic plant data set was used to estimate the total number and location of symptomatic plants ($y$) in plots. The incidence ($\bar{y}$) was calculated as $\bar{y} = y_i / n_i$, where $y$ was the number of diseased plants and $n$ the estimated number of total plants (asymptomatic and symptomatic) in the $i^{th}$ plot. The incidence was calculated at each assessment date. The Monomolecular, Logistic and Gompertz models were fit to the disease incidence data and fitted over time by nonlinear regressions using the nlsLM function from MINPACK.LM package (Elzhov et al. 2016). The choice of the best model was performed by linear mixed-effects model (Laird and Ware 1982) using the LME function from NLME package (Pinheiro et al. 2018).

*Spatial analysis*. Spatial analyses were performed using quadrat- and distance based methods. The symptomatic plant data set was used for quadrat-based analyses, in which the plot maps were *quadratized* using the QUADRATCOUNT function of the SPATSTAT package (Baddeley and Turner 2005). The quadrat size used was 2 x 2, i.e. the area occupied by two plants within and between rows, respectively. The number of symptomatic plants in each quadrat was determined and used to perform the spatial analysis. The exact number of plants inside each quadrat could not be

computed because the direction of rows and spacing between plants was irregular. The estimated precision of the GPS device varied between 3 and 7 m during the assessments depending on the environmental conditions.

Dispersion index for binomial data, $D$, referred sometimes as the ratio between variances was used for a quadrat-based method. To test the null hypothesis for dispersion index ($D$ = 1) a $\chi^2$ test was performed. The analysis was conducted using the AGG_INDEX function from the EPIPHY package (Gigot 2018). Spatial Analysis by Distance IndicEs (*SADIE*) was the method chosen in the distance-based approach. *SADIE* uses the location of the sampling units (i.e. quadrats) and the number of individuals (i.e. symptomatic plants) inside the unit to analyze the spatial arrangement by the distance to regularity ($D_r$) criterion. $D_r$ is achieved when all the sampling units have the same number of diseased individuals. *SADIE* returns an overall aggregation index ($I_a$) which reflects the ratio between the distance moved to achieve a regular pattern for the observed data and a theoretical mean to regularity based on random permutations. The index developed by Li et al. (2012) was computed by the SADIE function from EPIPHY package. On these two methods ($D$ and *SADIE*) a regular pattern was inferred when the indices were equal to 1, an aggregated pattern when they were higher than 1, and regular when less than 1.

*Spatio-temporal analysis.* The relationship between the observed and theoretical variances of FW incidence per sampling unit was evaluated. The equation for binary power law, $\log_{10}(s^2) = \log_{10}(A) + b \log_{10}(s^2_{bin})$, where $s^2$ its the observed variance, $s^2_{bin}$ the theoretical variance assuming a binomial distribution, and $A$ ($\log_{10}(A)$) and $b$ are the parameters to be estimated. Estimates of $\log_{10}(A) = 0$ and $b$ = 1 suggest a random distribution, if $\log_{10}(A) > 0$ and $b$ = 1 suggest an aggregated pattern with fixed value independently of the incidence level; and if both parameters,

$\log_{10}(A)$ and $b$, are higher than 0 and 1, respectively, the higher the incidence the greater the values of the aggregation index (Madden and Hughes 1995). A t-test was performed to test the null hypothesis for individual plots, and to test if the parameters differed between the plots.

**Association between *Fusarium oxysporum* f. sp. *cubense* and weevil borer**

*Insect collection*. WB and FWB were collected in the same plots and in the same pseudostem traps used to monitoring. Collections were performed 14 days after setting the traps in the plot. Insects were collected four times in one year period, one in each climatic season, from June 2017 to June 2018. At each time, WB and FWB, were quantified and placed individually in microtubes properly identified.

*Fusarium* spp. *collection*. Microtubes containing the insects were filled with 300 µL of water containing streptomycin (1 g/L). The insects were washed following the procedures described by Meldrum et al. (2013) and the suspension was spread in plates containing Komada medium (Komada 1975) with 0.5% of lactic acid. Plates were maintained in growth chamber at 25 ºC, in dark conditions for seven days. Fungal colonies were quantified and a maximum of 5 per plate were selected and subcultured into potato-dextrose-agar (PDA). *Fusarium* spp. colonies were analyzed for morphological aspects under an optical microscope (400X; Olympus CX31) (Leslie and Summerell 2006). Isolates were transferred to SNA, maintained at 25 ºC for ten days, mycelial disks were transferred to microtubes and stored at 10 ºC and add to the *Ins*-UFV collection.

*DNA purification*. Total DNA was extracted from 370 isolates using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, United States of America) following the manufacturer's instructions with some modifications. Briefly,

191 dried mycelium was disrupted for 2 min using TissueLyzer II (Qiagen, Valencia, CA,
192 USA). After the nuclei lysis and protein precipitation steps the supernatant was
193 transferred to a clean microtube containing 300 µL of 2% mixed alkyl trimethyl
194 ammonium bromide (MATAB) and 300 µL of chloroform-isoamyl alcohol (24:1
195 v/v) and vortexed at high speed for 20 s. Tubes were centrifuged at 14,000 rpm for
196 10 min and the supernatant was collected. The remainder of the DNA extraction was
197 performed according to the manufacturer's instructions. DNA integrity was analyzed
198 using electrophoresis on a 0.8% agarose gel in TBE (0.089M Tris base, 0.089M
199 Boric acid, 0.002M EDTA pH 8.0). DNA quality and quantity were determined
200 using NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA,
201 USA).

202 *Pathogen detection*. Foc-UFV primers (forward and reverse) were developed
203 to amplify a conserved region of 122 bp of the genome of *Foc* (confidential data, *in*
204 *development*). PCR reactions were performed in a volume of 12.5 µL, which
205 consisted of 2.5 µL of 5x GoTaq® Reaction Buffer (Promega), 0.25 µL of PCR
206 nucleotide (10 mM), 0.2 µL of the forward and reverse primers each (1 µM), 0.05 µL
207 GoTaq® DNA Polymerase (5u/µL), 1 µL template DNA (~ 10 ng/µL), and nuclease-
208 free water to 12.5 µL. The thermal cycler conditions used were as follows: initial
209 denaturation at 95 °C for 5 min, followed by 35 cycles of PCR amplification at 95 °C
210 for 30 s for denaturation, annealing at 63 °C for 30 s, extension at 72 ºC for 45 s,
211 with a final extension at 72 ºC for 5 min. Positive control consisted of substituting 1
212 µL of DNA from the insects isolates for the *Foc*-UFV581, isolated from a FW
213 symptomatic plant in the field. Negative control was used substituting the DNA for
214 nuclease-free water. PCR products were analyzed on a 1.7% agarose gel in TBE.

## RESULTS

### Comparative epidemiology

Management and monitoring of WB were carried out from May 2017 to February 2019. The overall number of FWB collected (median of 2.3 per trap) was higher than WB (median of 1.1 per trap) in each assessment ($P < 0.001$). The number of WB was lower in the plot where WB was managed with *B. bassiana* (median of 0.67 per trap) compared to the unmanaged plot (1.6 per trap) ($P < 0.001$) (Fig 1a). Management with *B. bassiana* did not affect ($P = 0.789$) the population of FWB in the same period (Fig 1b).

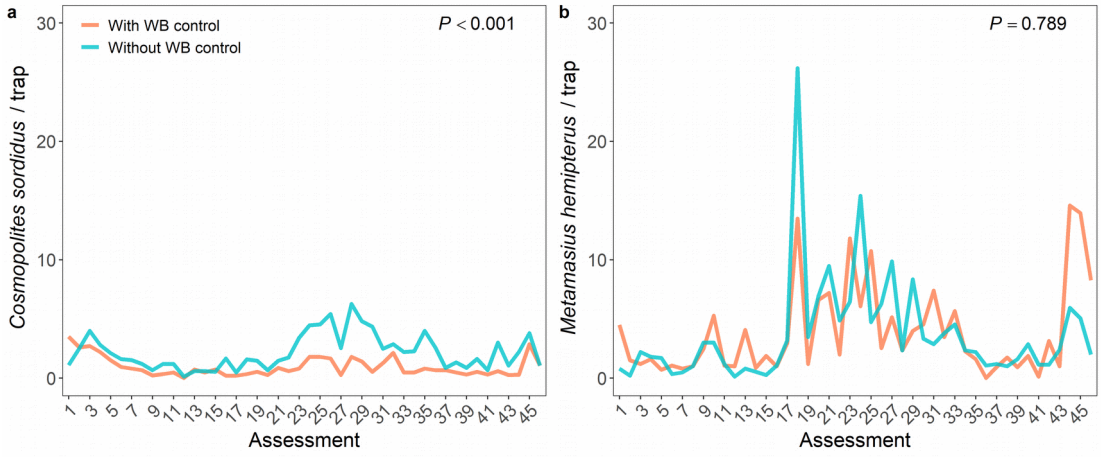
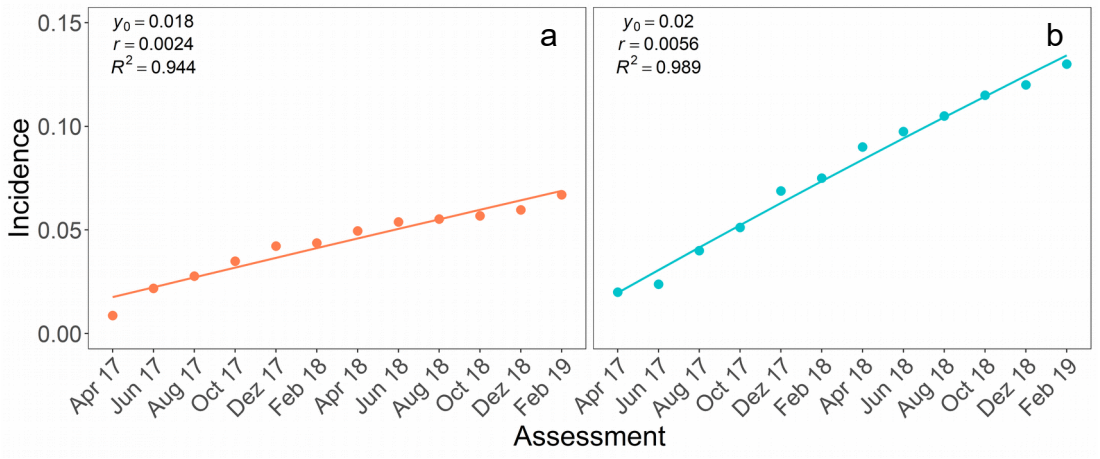



Fig 1. Biweekly monitoring of weevil borer (*Cosmopolites sordidus*, WB) (a); and false weevil borer (*Metamasius hemipterus*, FWB) (b) in fields with and without WB control, from May 2017 to February 2018, assessments 1 to 45, respectively.

Fusarium wilt incidence was lowest in first assessment with 0.9% and 2% in the plot with and without WB management, respectively (Fig 2). These two plots already had diseased plants when the assessments began. After 12 assessments, disease incidence reached 6.7% and 13% in plots with and without WB management, respectively (Fig 2). Monomolecular model best described the disease progress in

232 both plots (Table 1). Initial incidence did not differ for the two plots studied and the
233 disease rate was higher in the plot without management of WB ($P < 0.001$) (Fig 2).




234 Fig 2. Incidence of Fusarium wilt of banana in plots with (a) and without (b)
235 management of weevil borer (*Cosmopolites sordidus*, WB) from April 2017 to
236 February 2019 (points). Monomolecular model adjusted to incidence data (curves)
237 The plots were located in Teixeiras, Minas Gerais state, Brazil. Estimated initial
238 incidence ($y_0$), progress rate ($r$) of epidemics and the measure of the quality of the
239 adjusted monomolecular model ($R^2$) are presented.

240 Table 1. Summary of statistics used to evaluate the progress of Fusarium wilt of
241 banana in two plots, with and without weevil borer management, located in
242 Teixeiras, Minas Gerais state, Brazil, from April 2017 to February 2019.

| Model | AIC | BIC | logLikelihood | Best adjust / *n* |
|---|---|---|---|---|
| Monomolecular | -219.95 | -213.98 | 115.98 | 2/2 |
| Logistic | -137.39 | -131.41 | 74.69 | 0/2 |
| Gompertz | -153.44 | -147.47 | 82.72 | 0/2 |

243 AIC: Akaike Information Criterion; BIC: Bayesian Information Criterion.

Spatial analysis were computed for both plots in quadrat- and distance-based methods. FW aggregation was inferred based on dispersion index in all assessments in both plots (Fig 3a). Aggregation was lowest in the first two assessments and highest in the last. Aggregation was higher in the plot with management of WB and $D$ ranged from 1.90 to 2.47 with a mean of 2.23 than in the unmanaged field ($P <$ 0.001). In the plot without management, $D$ ranged from 1.34 to 2.27 with a mean of 1.86 (Fig 4a). Random pattern was detected using *SADIE* in the first two assessments only when the $I_a$ values were low (1.15 and 1.23) (Fig 4b). The highest $I_a$ value (2.34) was observed in the plot with management of WB. The degree of aggregation obtained by *SADIE* differed between the plots: aggregation index was higher in the managed (2.04) compared to the plot without WB management (1.82; $P = 0.016$; Fig 4b).

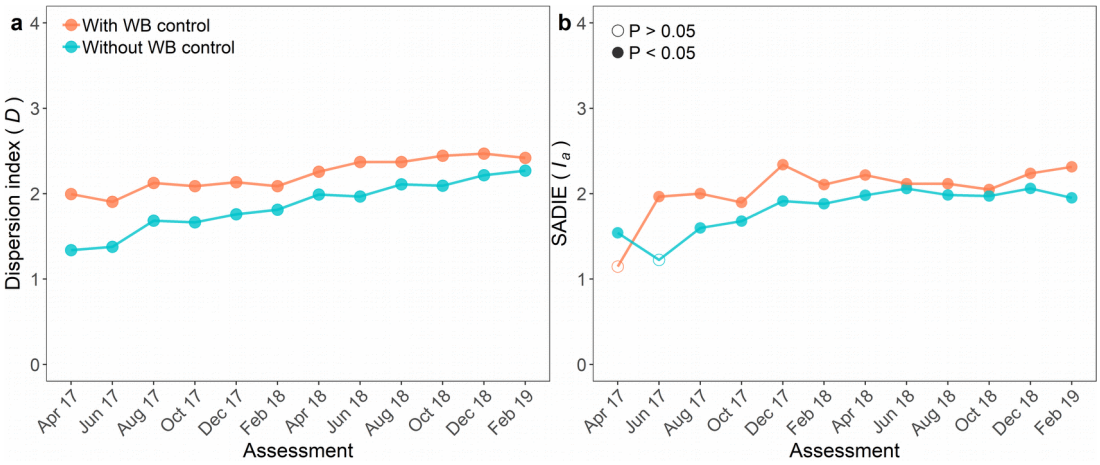



Fig 4. Dispersion index ($D$; a) and aggregation index ($I_a$) of Spatial Analysis by Distance Indices (*SADIE*; b) of incidence of Fusarium wilt of banana in two plots, with and without management of weevil borer (*Cosmopolites sordidus*, WB), from April 2017 to February 2019. The plots were located in Teixeiras, Minas Gerais state, Brazil. $P$-value was represented by empty ($P > 0.05$) or filled ($P < 0.05$) symbols.

Binary power law express the relationship between the logarithm of observed variance and logarithm of theoretical variance assuming a binomial distribution, and was well adjusted in plots with ($R^2$ = 0.992) and without ($R^2$ = 0.998) WB management (Fig 5). Parameters for power law, $A$ and $b$, were significantly ($P$ < 0.001) different 1 for both plots. Aggregated patterns that increased with incidence were observed. $Log_{10}(A)$ and $b$ also differed between plots ($P$ < 0.001). The plot without WB management had higher degree of aggregation ($log_{10}(A)$ = 0.66) than the plot with management and was strongly ($b$ = 1.29) affected by the increase of incidence over time (Fig 5).

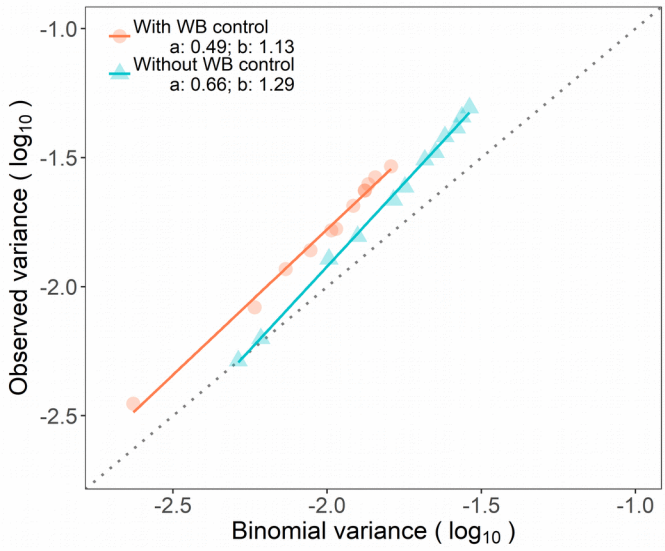



Fig 5. Binary power law of successive assessments for incidence of Fusarium wilt of banana from April 2017 to February 2019 in two plots, with and without management of weevil borer (*Cosmopolites sordidus*, WB). The plots were located in Teixeiras, Minas Gerais state, Brazil.

**Association between *Fusarium oxysporum* f. sp. *cubense* and weevil borer**

A total of 115 WB and 102 FWB were collected from April 2017 to February 2019. All of them were washed and a total of 9,347 colony forming units (cfu) were formed in selective medium for *F. oxysporum*. Colony forming units were higher in WB (median = 21) than FWB (4) ($P < 0.001$) (Fig 6).

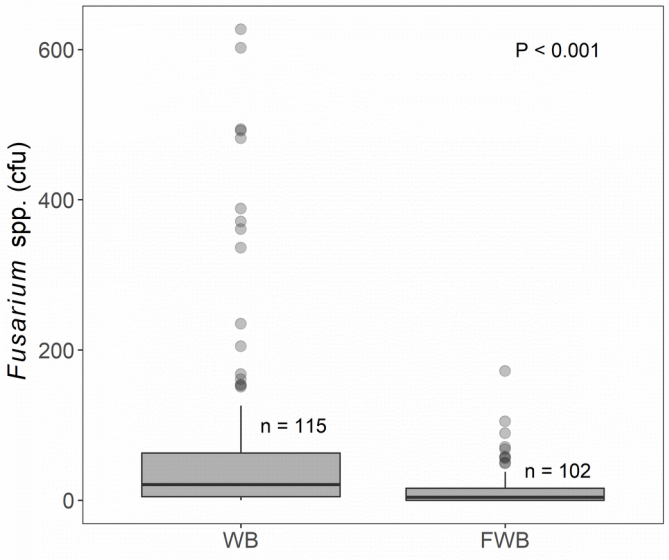



Fig 6. Number of colony forming units (CFU) in selective medium for *Fusarium oxysporum* associated to weevil borer (*Cosmopolites sordidus*; WB) and false weevil borer (*Metamasius hemipterus*; FWB).

From the total colonies observed, 370 subcultures were selected, purified and analyzed by PCR, but none was *Foc* (Fig 7). Morphological characterization was performed for 154 strains and 61% ($n = 94$) were classified as *F. oxysporum*.

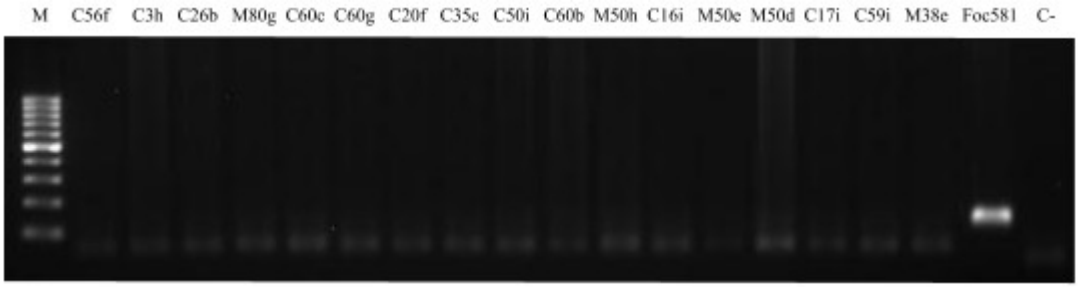



Fig 7. PCR analysis using *Foc*-UFV primers to amplify a conserved region of 122 bp of 17 *Fusarium* spp. strains associated with weevil borer collected in fields with epidemics of Fusarium wilt. M is a 100pb marker. Lanes 2 to 18 are PCR products from weevil borer (*Cosmopolites sordidus*; code starts with C) and false weevil borer (*Metamasius hemipterus*; code starts with M). Foc581 refers to a positive control (pathogenic strain) collected from diseased plant in the same field and stored at *Foc*-UFV collection with code *Foc*-UFV581. Negative control is identified as C-.

## DISCUSSION

Currently, there is high interest in the dispersal mechanisms of *Foc* (Dita et al. 2018; Ploetz et al. 2015). This information is crucial for management of FW in already infested areas as well as for setting exclusion and mitigation actions in disease-free fields. The role played by pests that are present in banana fields still needs to be elucidated. Epidemiological information can be useful to develop management strategies to effectively reduce the spread of *Foc* and reduce the damage caused by FW. In this study, two approaches were used to study the role of the vector on disease development: a comparative epidemiology study under field conditions; and an association study involving the capture of insects potentially involved in *Foc* transmission.

304 The management of banana weevil (*C. sordidus*) was performed using *B.*
305 *bassiana* an entomopathogenic fungus widely used to control banana weevils and
306 FWB (Akello et al. 2008; Kaaya et al. 1993; Mesquita et al. 1981; Pauli et al. 2011).
307 The fungus enter the insect through the spiracles, digestive system or insect cuticle
308 and grows in the haemocoel and muscle tissues, destroys tracheal taenidia and fat
309 bodies (Kaaya et al. 1993). In both plots, the population of FWB was higher than that
310 of WB. False weevil borer is considered a secondary pest in banana and other crops
311 (Fancelli et al. 2012). However, in high population it damages the banana plantations
312 and management needs to be considered (Fancelli et al. 2012). In this study, the
313 management did not affect the population of *M. hemipterus* even though many
314 infected individuals were found in traps after the application of *B. bassiana*. An
315 abandoned banana plantation close to the plot with management probably masked the
316 counts of FWB in the traps due to their high mobility. This could have increased the
317 chance of FWB to be attracted to the pseudostems traps (Dolinski and Lacey 2007).
318 Differences in the dynamic of FW incidence could be seen between the two
319 plots. The incidence was lowest in the first assessment and increased over time,
320 reaching up 13% in the plot without WB management and reached 6.7% in the plot
321 with control of WB. These values can be considered low when compared with
322 reports in Tanzania and Indonesia where the incidence reached up to 77% and 100%,
323 respectively (Hermanto et al. 2011; Karangwa et al. 2016). However, the stage of
324 FW epidemics, cultivars, environmental conditions and cultural practices could affect
325 the incidence observed. The plots studied were cultivated with a Prata type cultivar
326 (AAB genome) that shows intermediate resistance to race 1 of the pathogen when
327 compared to Gros Michel or Silk subgroups. This fact probably reduced the disease
328 progress (Dita et al. 2018). FW progress was described with the monomolecular

329 model. Differences between the initial incidences were not detected and the disease
330 rate was higher in the plot with higher population of WB. This fact indicated that the
331 dynamic of FW epidemics may have been affected by the insect population, probably
332 acting as dispersal agent or predisposing the banana plants to infection by *Foc*
333 (Meldrum et al. 2013).

334 Both fields showed an aggregated pattern during the study, but a higher
335 degree of aggregation was observed in plot with lower populations of WB. Higher
336 spatial heterogeneity was observed in the plot with management of WB when
337 compared with the plot without management. The higher degree of aggregation could
338 be assigned to the plant-to-plant transmission. A diseased plant acts as an inoculum
339 source to the closest plants, i.e. the neighbours plants. Root-to-root was the main
340 dispersion way of *F. oxysporum* f. sp. *radicis-lycopersici* in tomato fields (Rekah et
341 al. 1999). The same pattern was observed by the distance-based method, *SADIE.*
342 The clusters of diseased plants were closest in WB managed field than in
343 unmanaged. In addition, the degree of aggregation increased over time showing
344 higher values in the last assessments for both, $D$ and $I_a$. Initial infections of stem
345 bleeding disease of coconut palm had a random pattern in the first assessments, but
346 evolved to aggregated pattern in the last (Carvalho et al. 2013).

347 In the spatio-temporal approach, at low levels of incidence, the plot managed
348 for WB presented higher aggregation of FW. However, as incidence increases, the
349 level of aggregation increases at higher rates in the plot without management.
350 Probably, reducing the population of a potential vector, WB, the pathogen dispersal
351 at greater distances would be reduced and the nearby plants get infected more easily.

352 It is important to highlight that the behaviour of WB and FWB presented
353 great differences that can impact the spatio-temporal dynamic of FW epidemics. *C.*

354 *sordidus* adults have limited mobility. Even though they have functional wings, flight
355 is rare, movement by crawling is limited and attracted by host volatiles (Gold et al.
356 2001). Unpublished studies performed in Uganda demonstrated that 81% of the *C.*
357 *sordidus* released moved a maximum of 15 m in six months, and only 3% moved
358 more than 25 m (Gold et al. 2001). On the other hand, the movement of *M.*
359 *hemipterus* is much more common, they are good flying insects and are attracted by
360 wounds and plant debris, but can feed in healthy banana plants in higher populations
361 (Fancelli et al. 2012; Molet 2013). Although WB had limited movement, differences
362 in spatial patterns were detected between the two fields studied and could bring new
363 insights about the impact of these two pests in FW epidemics.

364 In this study a great number of *Fusarium* spp. were externally associated with
365 *C. sordidus* and *M. hemipterus*. The number of *Fusarium* spp. associated with WB
366 was higher than FWB. Many species of *F. oxysporum* are soilborne fungi (Leslie and
367 Summerell 2006) and due to the crawling habit of WB (Gold et al. 2001), soil
368 particles can easily be found in their exoskeleton. As FWB is a good flier, probably
369 their movement in soil is not as common as WB (Molet 2013). In this way, it was
370 expected that WB, at individual level, had higher potential to carry *Fusarium*
371 propagules than FWB. In addition, it is hypothesized that WB, as larvae have a
372 preference to feed in rhizome (Gold et al. 2001) while larvae of FWB prefer to feed
373 in stems or plant debris (Fancelli et al. 2012; Molet 2013), thus the quantity of
374 *Fusarium* propagules carried by WB may be higher. Further studies needs to be
375 performed to test this hypothesis.

376 PCR amplifications using *Foc*-specific primers were not positive for any of
377 the collected and purified *Fusarium* spp. isolates. Meldrum et al. (2013) identified
378 ten percent of the WB collected in fields with FW epidemics carrying viable spores

379 of *Foc* TR4. *Fusarium oxysporum* f. sp. *cucumerinum* spores were found externally
380 on the bodies of sciarid and shore flies in concentration that reached up 1 x $10^6$
381 copies per individual artificially infested (Scarlett et al. 2014). The transmission was
382 confirmed by pathogenicity tests in greenhouse trial (Scarlett et al. 2014). Although
383 no *Foc* was detected, microscopy analysis identified approximately 61% of the
384 strains (n = 154) belonging to *F. oxysporum* species. The fact that the pseudostem
385 traps were previously distributed in the plot at random, only one (out of 30) was
386 ended up placed near to a symptomatic plant. Therefore, the fact that the majority of
387 traps were located near by asymptomatic plants could have reduced the probability to
388 associate *Foc* with WB. In addition, less than 4% of the total CFU grown in selective
389 media for *F. oxysporum* were analyzed by PCR assay or microscopy.
390 This study showed a potential impact of the WB population in the spatio-
391 temporal dynamic of FW epidemics under field conditions. Fields with lower WB
392 population presented a more aggregated pattern, demonstrating that dispersal of *Foc*
393 at greater distances was minimized. *C. sordidus* and *M. hemipterus* were able to
394 carry viable propagules of *Fusarium* spp. on the external surface of their bodies.
395 These results may direct new studies to clarify the interaction between *Foc* and their
396 potential vectors. A better management to FW of bananas depends on the
397 development of efficient strategies to reduce the dispersal of *Foc* in the fields.

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# GENERAL CONCLUSIONS

Fusarium wilt incidence in Brazil is moderate (~11%) and is higher in fields planted with Silk (14.3%) than Pome (4.4%) and Cavendish (2.7%) subgroups.

The epidemics of FW in Brazil can be considered as monocyclic, develop mostly in an aggregated pattern at small-scale level of observation, but clusters of diseased plants were randomly distributed in most plots. Additionally, FW seems be primarily affected by cultivar, nutritional state of the plants and soil physical factors.

Aerial dispersal of *Fusarium oxysporum* f. sp *cubense* was confirmed.

Weevil borer (*Cosmopolites sordidus*) and false weevil borer (*Metamasius hemipterus*) can carry propagules of *Fusarium* spp. on the external body surface. Weevil borer affects the spatio-temporal dynamics of FW epidemics. In fields with high populations of the insect, FW epidemics developed at higher rates and aggregation was more evident.