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Effect of environmental conditions on spore production by *Fusarium verticillioides*, the causal agent of maize ear rot

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Abstract Silk infection by *Fusarium verticillioides* is caused by conidia produced on maize crop residues and results in kernel infection and consequent accumulation of fumonisins. Studies were carried out in both controlled and field conditions to understand the dynamics of sporulation on maize residues. The effect of temperature (5°C to 45°C) and incubation time (3 to 41 days) on spore production on maize meal agar was described by a logistic model that accounted for 85% of variability. The rate parameter depended on the length of incubation and the asymptote on temperature. Maximum sporulation occurred at 27°C, with a progressive increase between 5°C and 27°C and then a rapid decline, with no sporulation at 45°C. *Fusarium verticillioides* strains from different geographic origins showed different sporulation capabilities, with similar optimum temperatures. Pieces of stalk residues inoculated with *F. verticillioides* and placed above the soil between rows of maize crops, in 2003 to 2005, produced conidia continuously and abundantly for some weeks, particularly during the period after silk emergence, with an average of 1.59×10^7 conidia g^{-1} of stalk, over a wide range of environmental conditions. Sixty-seven percent of variability of the spore numbers found on stalks was accounted for by a multiple regression model. Precipitation (rain or overhead irrigation) in the 14 days before stalk sampling decreased the number of spores, whilst the number of days with conducive conditions of moisture (i.e. days with rainfall, average relative humidity >85% or vapour pressure deficit <4 hPa) and greater degree-days (base 0°C) in the 14 and 3 days before sampling, respectively, increased sporulation.

Keywords Available water · Inoculum sources · Maize crop residues · Weather variables

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Introduction

Fusarium verticillioides (synonym *F. moniliforme*) (teleomorph: *Gibberella moniliformis*) (synonym *G. fujikuroi*) is an important pathogen of maize. It causes maize ear rot, but is mainly considered for its ability to produce fumonisins (FBs), mainly B_1 and B_2. These toxins cause equine leukoencephalomalacia in horses, pulmonary oedema in swine (Colvin and Harrison 1992), hepatotoxic and nephrotoxic effects in other domestic animals, carcinogenesis in laboratory animals and have been associated with oesophageal cancer of humans in certain areas of the world (Marasas 2001).

An important tool to manage FBs in maize would be the use of a simulation model that is able to predict fungal development and toxin production based on weather conditions (Munkvold 2003). Environmental factors that may influence *Fusaria* causing head

blight of wheat have been extensively studied (see reviews by Parry et al. 1995; Xu 2003) and the results incorporated in empiric models which assess the risk of infection and mycotoxin production (Del Ponte et al. 2004), and in a mechanistic model that simulates the life-cycle of these fungi (Rossi et al. 2003).

Battilani et al. (2003) developed a conceptual model simulating the life-cycle of *F. verticillioides* in maize and pointed out that more information is needed to develop a model for use in practice. Therefore, efforts are required to understand the epidemiology of this disease by focusing more precisely on the relationship between weather variables and specific components of the disease-cycle, including the production of inoculum for silk infection (Munkvold 2003).

It has been demonstrated that maize crop residues are the primary source of inoculum, because *F. verticillioides* survives in these residues for a long time (Nyvall and Kommedahl 1970; Cotten and Munkvold 1998), and it was also assumed that the fungus may form large numbers of microconidia and macroconidia on these residues during the season (Munkvold 2003). However, the effects of environmental conditions on the sporulation dynamics on maize crop residues are not yet known. Infected tassels can also act as a source of inoculum for silk infection, but their role can be considered of secondary importance (Logrieco and Bottalico 1987).

The aim of this study was to (1) study the dynamics of *F. verticillioides* sporulation at different temperature regimes and length of incubation, and (2) quantify sporulation on maize stalk residues under field conditions, in relation to meteorological variables.

Materials and methods

Fungal strains

Two strains of *F. verticillioides*, ITEM 1744 and ITEM 1773, both isolated from maize kernels and confirmed to produce fumonisins (Moretti et al. 1995) were kindly provided by the Institute of Sciences and Food Production (ISPA-CNR, Bari, Italy). Other strains were isolated from maize kernels collected in commercial crops grown in four regions in northern Italy (Piedmont, Lombardy, Veneto, and Friuli Venezia Giulia) in 2002.

For fungal isolation, kernels were washed in running tap water for 20 min, sterilised with ethyl alcohol (90%) for 2 min and then in sodium hypochlorite (0.8%) for 2 min, rinsed three times in sterile water and dried on absorbent paper under a sterile hood. Kernels were placed in Petri plates containing Peptone PCNB Agar (PPA; 15 g l^{-1} peptone, 1 g l^{-1} KH_2PO_4, 0.5 g l^{-1} $MgSO_47H_2O$, 1 g l^{-1} PCNB 75%, 8 g l^{-1} agar), at 25°C. After 7 days of incubation, the fungal colonies were transferred on Potato Dextrose Agar (PDA; 1l potato broth 600 g l^{-1}, 10 g l^{-1} sucrose, 15 g l^{-1} agar). Single-spore strains were obtained from these colonies, cultivated on Carnation Leaf Agar (CLA; 15 g l^{-1} agar, sterilised carnation leaves), identified according to Leslie and Summerell (2006), and stored at 4°C in the fungal collection of the Institute of Entomology and Plant Pathology, Università Cattolica del Sacro Cuore. Fungal identification was confirmed by Dr. Antonio F. Logrieco (ISPA-CNR, Bari, Italy).

To perform the experiments, the fungal strains from the fungal collection were grown in Petri plates (9 cm diam) on PDA, and incubated at 25°C for 15 days, with a 12 h day-light photoperiod.

Effect of temperature, incubation time and strains on spore production

The effect of temperature and the length of incubation time on in vitro spore production was determined using Petri plates inoculated with the strain ITEM 1744. To avoid interactions between temperature, spore production and colony growth, all the Petri plates used in this experiment were prepared as follows. Mycelial plugs (0.5 cm diam) were taken from the margin of fungal colonies grown on PDA as previously described and placed in the centre of Petri plates (9 cm diam) containing 3% Maize Meal Extract Agar (MMEA), adjusted to pH 5.5 (Marin et al. 1998). All the plates were first incubated at 15°C until the colonies had uniformly covered the plate surface. Three plates were then used to determine spore production at the beginning of the experiment (time zero), while the other plates were incubated for 3, 6, 9, 15, 21, 31, or 41 days at 5°C to 45°C (step 5°C) under a 12 h day-light photoperiod. There were three replicate plates per incubation time × temperature combination. To collect spores, 5 ml of bi-distilled water was added to each plate and the colony surface

was gently scraped. The resulting suspension was filtered with a sterile double gauze layer and the number of conidia counted on three replicated aliquots of 0.2 ml using a Burker chamber (9 counts in total per treatment=3 aliquots×3 plates). Spore production was then expressed as number of conidia per square centimeter by subtracting the number of spores produced at time zero. The same procedure was used for the 16 other strains from different geographic locations, but they were incubated only for 15 days at 25°C, 30°C or 35°C. The experiments were repeated once.

Spore production on inoculated maize stalks in field

The maize hybrid PR33J24 (Pioneer Hi-bred Inc., Des Moines, Iowa, USA), FAO class 600, was grown in Piacenza (northern Italy) in 2003, 2004 and 2005, on a loam-clay soil. The crops were managed according to the ordinary cropping system for the area, with a density of seven plants per square meter. Neither pesticides nor fungicides were sprayed during the growing season. The crop was irrigated following the common practice, using an overhead high-pressure sprinkler with a maximum flow of 1,300 l of water min^{-1} (mod. L, Casella Macchine Agricole S.r.l. Piacenza, Italy). Air temperature, relative humidity and rain were recorded hourly by an automatic station (mod. AD2, Silimet S.r.l. Modena, Italy) placed about 1 km from the experimental plots.

Residues of maize stem pieces were collected in the field about 15 days after harvesting in 2002, 2003 and 2004. They were stored at 5°C until next June when they were cut into small pieces, 5–6 cm in length, washed with running tap water, dried at room temperature for 24 h, and sterilised twice at 120°C for 20 min to eliminate any natural contamination by *F. verticillioides*. Sixty stalk pieces were then inoculated by spraying 3 ml of a conidial suspension of *F. verticillioides* prepared as follows. Fungal colonies of the strains ITEM 1744 and ITEM 1773, grown on PDA as previously described, were gently washed with 10 ml of sterile water; the resulting suspension was filtered with sterile gauze and adjusted to 2×10^8 conidia ml^{-1} using a Burker chamber. The two strains were used in mixture.

After inoculation, stalk pieces were arranged in metal net boxes (10×15×3 cm), one piece per box, enclosed in plastic bags to maintain 100% relative humidity, and incubated at 30°C for 20 days, under a 12 h day-light photoperiod. At the end of this period the stalk pieces appeared uniformly colonised by the fungus. Sixty of these boxes were placed on the soil of the maize crop each year at silk emergence (stage 61 of the BBCH scale) between plant rows. Three boxes were randomly collected at 3 to 4 day intervals between 11 July and 12 August 2003, 22 July and 23 August 2004, 22 July and 5 September 2005, and taken to the laboratory. Each stalk piece was weighed individually and placed in a beaker (500 ml); 200 ml of sterile distilled water was then added and the beaker was shaken for 10 min. *Fusarium verticillioides* conidia were counted using a Burker chamber on two replicate drops (100 μl per drop) (6 counts in total per treatment=2 drops×3 boxes), and expressed as conidia per gram of stalk piece.

In 2003, 70 additional boxes were placed as previously described but sheltered with a plastic cover placed about 1 m above the soil to protect stalks from rainfall. These stalks were sampled at the same time as the uncovered ones.

In 2004, 20 additional boxes with stalk pieces not inoculated were prepared, exposed in the field and sampled at 3 to 4 day intervals. The water activity (a_W) of these stalk pieces was measured in triplicate using a Hygroskop-BT (Rotronic Instrument Corp., New York, USA).

Data analysis

The analysis of variance (ANOVA) was applied to calculate the percent of variance in spore production accounted for by the different sources of variability used in the first (temperature, incubation time and their interaction) and in the second (fungal strain, temperature, and their interaction) laboratory experiment. Data (spore number) were transformed using the natural logarithm before ANOVA to reduce heterogeneity in variance.

Quantitative relationships between spore numbers in the first experiment, temperature and the length of incubation time were then analysed using regression analysis. Relative spore production (RSP, in a zero to one scale) was calculated by dividing the average number of spores counted in each 'temperature × incubation time' combination by the maximum found in each experiment, which was 9.22×10^7 spores cm^{-2} of fungal colony found after 15 days of incubation at

30°C. For example, the spores found after 21 days of incubation at 10°C were 5.23×10^7, and the correspondent RSP was 0.567 ($5.23\times10^7/9.22\times10^7$).

Several nonlinear regression models were fitted to the observed RSP data. The equation parameters were estimated using the nonlinear regression procedure of SPSS (ver. 13.0, SPSS Inc., Chicago, USA) which minimises the residual sums of squares using the Marquardt algorithm. The best model was chosen based on the adjusted R^2, the number of iterations taken by the Marquardt algorithm to converge on parameter estimates, magnitude of the standard error of the parameters, and magnitude and distribution of the standardised residuals (Clewer and Scarisbrick 2001).

Data concerning the amount of spores produced on each sample of stalk residues that were inoculated with *F. verticillioides* and kept under field conditions in 2003 to 2005 (35 samples in total) were transformed using the natural logarithm and regressed on other variables. This analysis was performed using a stepwise procedure to select the most relevant variables within a set of independent variables, which included: number of days since the previous sample; conidia found in the previous sample; average temperature (T, °C), degree-days (DD, base 0°C), total rainfall (R, mm), average relative humidity (RH, %), and vapour pressure deficit (VPD, calculated from T and RH following Buck (1981), measured in hPa) calculated over 3, 7 and 14 days before sampling. In addition, each of these variables was squared and cubed for inclusion in regression analysis. The number of days with favourable conditions of T (i.e. $25\leq T<35$°C) and moisture (i.e. days with $R>0$, RH>85% or VPD<4 hPa) were also calculated for the same 3-, 7-, and 14-day periods. Three dichotomic variables were also included to account for the effect of the year.

Water availability of the maize stalks exposed in the field was not included in the regression analysis because it was measured only in 2004. Nevertheless, relationships between a_W and the weather variables were analysed by calculating the Pearson's coefficients of correlation.

The numbers of spores found in the samples collected in 2003 were compared with those found in the stalk pieces protected from rainfall with the plastic covers, using the t-test on the ln-transformed data; between-sample variability of these data was calculated as coefficient of variation (CV, calculated as: (ds/average)×100, where ds is the standard deviation).

Results

Effect of temperature and incubation time on spore production

Temperature and incubation time, as well as their interaction, significantly ($P<0.0001$) affected the number of *F. verticillioides* conidia produced in vitro. Temperature was the most influential factor and accounted for about 82% of the total variance, while incubation and the interaction accounted for 10% and 4%, respectively. After 3 days of incubation, conidia were observed at all temperatures considered, but at very low levels at 5°C, 40°C and 45°C (Fig. 1); the highest spore production occurred after 15 days of incubation at 30°C (9.22×10^7 spores cm^{-2} of colony).

The best fit of the RSP was obtained using the following regression equation:

$$\mathrm{RSP} = \left(a + b\times T + c\times T^2 + d\times T^3\right)/[1+\exp(e - f\times t)] \quad (1)$$

where: T is temperature (in °C); t is the incubation time (in days); a to f are the equation parameters.

The standard errors of the equation parameters were low compared to the parameter estimates, the adjusted R^2 was 0.85 (Table 1) and the plot of estimated versus observed values did not show

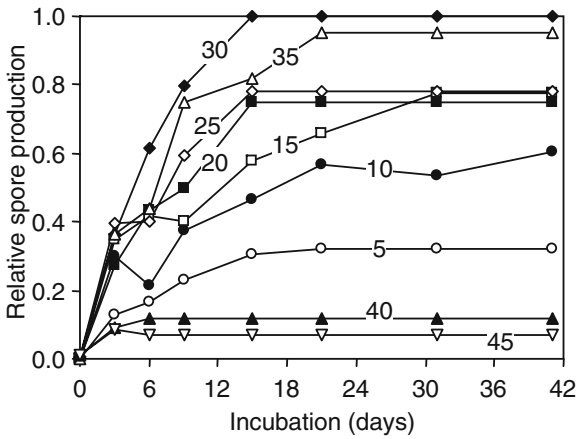

Fig. 1 Relative production of *F. verticillioides* conidia on Maize Meal Extract Agar at different temperatures (5°C to 45°C) and incubation times. Relative production is calculated by dividing the number of conidia counted in each combination of 'temperature × incubation time' by the maximum number found in the experiment (each point is an average of nine counts)

Table 1 Parameter estimates and summary statistics of nonlinear regression analysis relating the relative production of *F. verticillioides* conidia to temperature and incubation time

Parameter[a]	Estimate	SE[b]	R^2_{adj}	SE_{est}
a	+0.184	0.128	0.85	0.017
b	+0.025	0.020		
c	+0.00116	0.00095		
d	−0.00004	0.00001		
e	+1.795	0.358		
f	+0.333	0.065		

[a] Regression equation is: $RSP = (a + b \times T + c \times T^2 + d \times T^3)/[1 + \exp(e - f \times t)]$, where: RSP is relative spore production (0–1) on Maize Meal Extract Agar, *T* is temperature (°C) and *t* is incubation time (days). RSP is calculated by dividing numbers of spores counted in each combination of 'temperature × incubation time' by the maximum number found in the experiment

[b] SE is the standard error of each parameter estimate; R^2_{adj} is the coefficient of determination adjusted for the number of parameters (n= 72); SE_{est} is the standard error of the model estimates

systematic deviations (Fig. 2). Based on this equation, the dynamics of spore production over time followed a logistic curve (Fig. 3). In this curve, the parameter *e* accounts for initial sporulation, which is equal for all the temperature regimes, the rate parameter *f* depends on time, while the asymptote (i.e. the numerator) depends on temperature, according to a third-order polynomial equation. Maximum sporulation is estimated to 27°C, with a progressive increase between 5°C and 27°C, a rapid decline when T>27°C, and no sporulation at 45°C (Fig. 3).

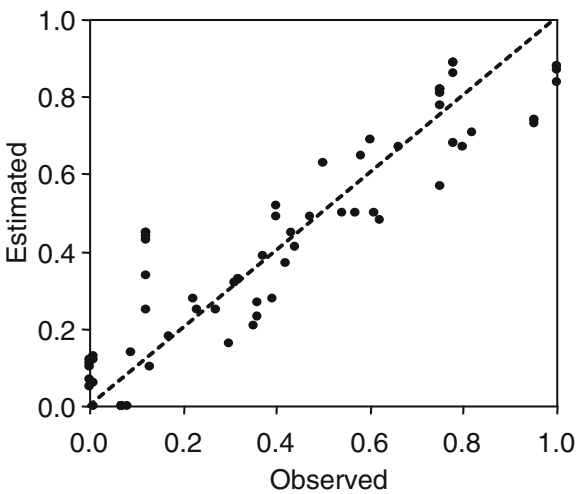

Fig. 2 Plot of estimated versus observed relative production of *F. verticillioides* conidia. Relative production is calculated by dividing numbers of conidia counted in each observation by the maximum number found in the experiment. Estimates are calculated by the regression equation of Table 1. The *dotted line* represents the coincidence of estimated and observed data

The effect of three temperature regimes (25°C, 30°C and 35°C) on spore production by the sixteen *F. verticilloides* strains of different geographic origins was significant (P<0.0001) and accounted for 36% of the total variance, but also strains (P<0.0001) and the temperature × strain interaction were significant (P= 0.0003) accounting for 39% and 22% of the total variance, respectively. Different strains showed varying sporulation capabilities (Table 2). The greatest number of spores were produced by strains 13, 15 and 16 (>7×10^7 conidia cm^{-2} of colony), but not significantly different from strains 1, 4, 8 to 10, and 14. Strain 5 was least productive (1.94×10^7 conidia cm^{-2}).

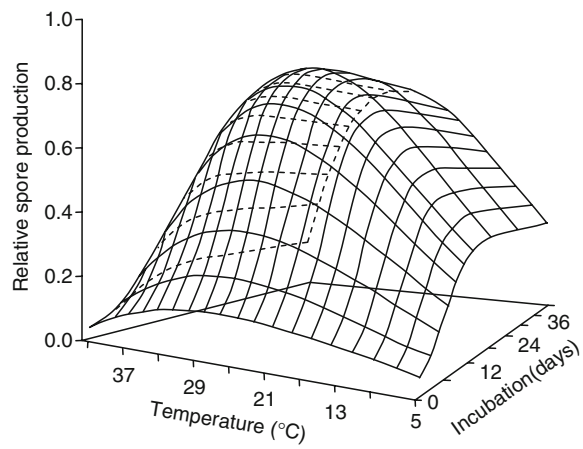

Fig. 3 Relationship between temperature, incubation time and relative production of *F. verticillioides* conidia as estimated by the regression equation of Table 1. Relative production is calculated by dividing numbers of conidia counted in each observation by the maximum number found in the experiment

Table 2 Production of conidia by 16 strains of *F. verticillioides* at different temperature regimes

Strain	Region[a]	Conidia[b]						Average	
		25°C		30°C		35°C			
1	Piedmont	5.05	*b*	7.48	*a*	2.68	*c*	5.07	a–d
2		4.42	*a*	5.20	*a*	1.87	*b*	3.83	c–e
3		2.90	*b*	5.28	*a*	0.78	*b*	2.99	ef
4		3.82	*b*	8.89	*a*	2.90	*b*	5.20	a–c
5	Lombardy	0.81	*b*	4.60	*a*	0.41	*b*	1.94	g
6		4.28	*b*	6.58	*a*	1.42	*c*	4.10	b–e
7		0.32	*b*	3.65	*a*	2.52	*a*	2.16	fg
8	Veneto	1.89	*c*	7.92	*a*	4.88	*b*	4.89	a–d
9		3.53	*b*	10.79	*a*	2.50	*b*	5.60	ab
10		4.70	*b*	7.17	*a*	2.50	*b*	4.79	a–d
11		1.40	*b*	7.61	*a*	1.96	*b*	3.66	de
12	Friuli	1.75	*b*	5.64	*a*	2.50	*b*	3.30	e
13		5.42	*b*	8.12	*a*	4.81	*b*	6.12	a
14		3.94	*b*	8.67	*a*	3.90	*b*	5.50	ab
15		7.49	*b*	9.74	*a*	3.20	*c*	6.81	a
16		7.02	*b*	11.09	*a*	2.41	*c*	6.84	a
Average		3.67	**b**	7.40	**a**	2.58	**c**		

[a] Region of origin of the maize kernels from which strains have been isolated

[b] Conidia are expressed as numbers ($\times 10^7$) cm^{-2} of the fungal colonies grown on Maize Meal Extract Agar for 15 days (averages of 3 replicate plates); numbers followed by the same letter are not significantly different using the Fisher Protected Least Square Difference Test (P=0.05) for each strain at different temperatures (in italics), over all strains between temperatures (in bold), and for the average of each strain (in normal characters); the analysis was performed on the ln-transformed numbers of conidia and the averages were back-transformed

In 16 out of 18 strains optimum temperature for sporulation was 30°C. In strain 2 spore production at 25°C and 30°C did not differ significantly, while in strain 7 production at 35°C was similar to that at 30°C (Table 3). Therefore, despite a significant interaction between strain and temperature, the response of these different strains to temperature was generally consistent.

Spore production on maize stalks in field

In 2003, stalk residues that had been inoculated with *F. verticillioides* were placed in the maize crop on 8 July, 6 days after silk emergence, and remained there for 35 days (Fig. 4a). This period was particularly hot and dry. The average air T over this period was 26.9°C,

Table 3 Parameter estimates and summary statistics of multiple regression analysis relating the production of *F. verticillioides* conidia on maize stalk residues in the field to a set of variables

Independent variables[a]		Unit	b_n	SE(b)[b]	$P(b)$	R^2_{adj}	SE_{est}
Intercept			16.17	0.907	<0.001	0.62	0.816
Days after exposure	X_1	No. of days	−0.070	0.012	<0.001		
Total rainfall in the previous 14 days	X_2	mm	−0.026	0.007	0.001		
Moist days in the previous 14 days	X_3	No. of days	0.276	0.067	<0.001		
Degree-days in the previous 3 days	X_4	Base 0°C	0.019	0.009	0.046		

[a] Regression equation is: $\ln(\text{conidia}) = b_0 + b_1 \times X_1 + b_2 \times X_2 + b_3 \times X_3 + b_4 \times X_4$

[b] SE(b) and $P(b)$ are standard errors and the probability levels of parameter estimates; R^2_{adj} is the coefficient of determination adjusted for the number of parameters (n=35); SE_{est} is the standard error of the model estimates

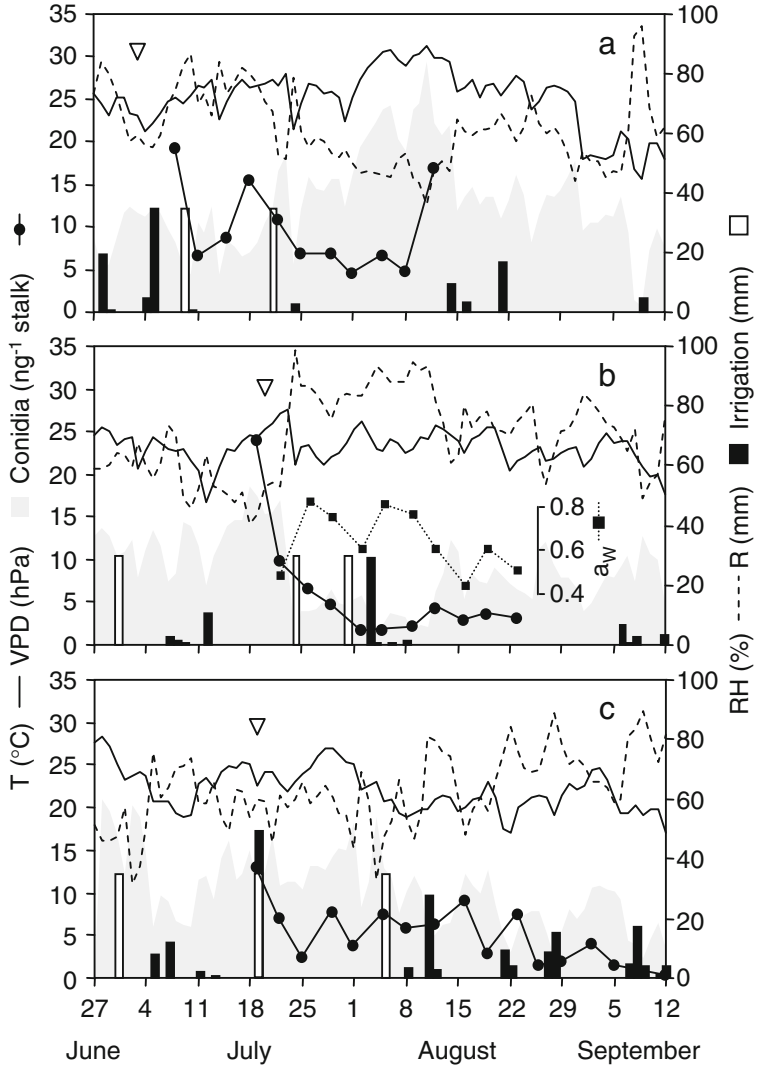

Fig. 4 Production of *F. verticillioides* conidia on maize stalk pieces placed on the soil between the rows of a maize crop in 2003 (**a**), 2004 (**b**) and 2005 (**c**), and corresponding temperature (*T*), relative humidity (RH), vapour pressure deficit (VPD), rainfall (*R*), overhead irrigation, and available water in stalk residues (a_W). Conidia are expressed as $n\times10^6$ (**a**, **c**) or $n\times10^7$ (**b**) g^{-1} of stalk (average of six spore counts). The *inverted triangle* symbol represents the beginning of silk emergence in the maize plants

with a minimum of 21.6°C and a maximum of 31.3°C, and average RH was 61.2% (36.2% to 86.8%), with only two rainy days (3.1 mm of rain in total). VPD was always higher than 4 hPa, with a maximum 29.5 hPa. The crop was irrigated overhead twice, on 9 and 21 July, each with 35 mm of water (Fig. 4a). A rainy period occurred in the 10 days before exposure of the stalks, with >60 mm of rainfall, so that the soil was moist at the time of stalk exposure.

The initial concentration of *F. verticillioides* conidia in the stalk pieces was 1.9×10^7conidia g^{-1} of stalk. Spore production decreased by 65% 3 days after exposure (6.6×10^6conidia g^{-1}) and after the irrigation of 9 July increased to 1.6×10^7conidia the following week. Afterwards spore production decreased until 8 August: during this period there was no rain and VPD was constantly high. A peak of spores was then observed on 12 August, with 1.7×10^7conidia, after a period with *T* of about 30°C.

In the stalk pieces protected from rainfall by the plastic covers the average number of conidia was 2.3 times higher than in the uncovered stalks and showed less between sample variability: the corresponding CV was 2.6% and 4.1%, respectively. This difference in spore number was significant at the *t*-test ($P<0.0001$).

In 2004, inoculated maize stalks were placed in the maize crop at the beginning of silking, on 19 July, and remained above the soil for 35 days (Fig. 4b). Average *T* of this period of exposure was 23.9°C (20.3°C to 27.5°C) and RH 78.3% (43.7% to 98.9%);

as a consequence the VPD was lower than in 2003, with an average 6.7 hPa (0.3 to 17.3 hPa), and there were some consecutive days with <4 hPa between 3 and 11 August. Rainfall was concentrated at the beginning of the period of stalk exposure, with 32.4 mm of rain in 6 days. The crop was irrigated twice each with 30 mm of water, on 24 and 31 July (Fig. 4b). The initial concentration of conidia was 2.4×10^8, decreased by about 60% after 3 days in the field (9.8×10^7conidia) and progressively decreased to $<1.6\times10^7$conidia after 21 days (on 5 August). Thereafter, spore production increased to 4.2×10^7conidia on 12 August, in a period with little rainfall and low VPD, and then remained at about 3×10^7conidia until the end of the exposure (Fig. 4b).

In 2005, the stalk residues were placed in the field on 19 July, at the beginning of silking, and were kept there for 55 days (Fig. 4c). Stalk exposure was followed by an irrigation of 35 mm of water and a rainfall of 10.9 mm; a further irrigation (35 mm of water) was applied on 5 August and thereafter there were regular rain events (12 rainy days with a total of 115.2 mm of water). The average T over the period of exposure was 21.7°C (17.0°C to 26.9°C), RH was 65.4% (33.1% to 89.7%), and VPD was 9.3 hPa (2.3 to 9.3 hPa). The initial concentration of conidia in stalk pieces was 1.3×10^7 conidia g^{-1}. The number of spores decreased by about 45% after 3 days (7×10^6 conidia) and then fluctuated between 1.4 to 8.9×10^6, with a minimum of 1.7×10^5 conidia in the last sample on 12 September (Fig. 4c).

A multiple regression model was derived to explain changes in spore number on maize stalk residues over time in term of weather conditions (Table 3). The stepwise regression selected included four variables, which accounted for 62% of the total variability. The first variable was the number of days after the inoculated maize stalks had been placed in the field; this variable had a negative regression coefficient, so that the production of spores decreased with time. Total precipitations (rain or overhead irrigation) in the 14 days before sampling had a negative regression coefficient, while the number of days with favourable conditions of moisture had a positive effect on sporulation, as well as the degree-days of the 3 days before sampling. The predicted spore concentration on maize stalks in the field (Fig. 5a) did not show systematic deviations and low residuals (all standardised residuals were within the interval ±2) (Fig. 5b), except the last sample of 2005.

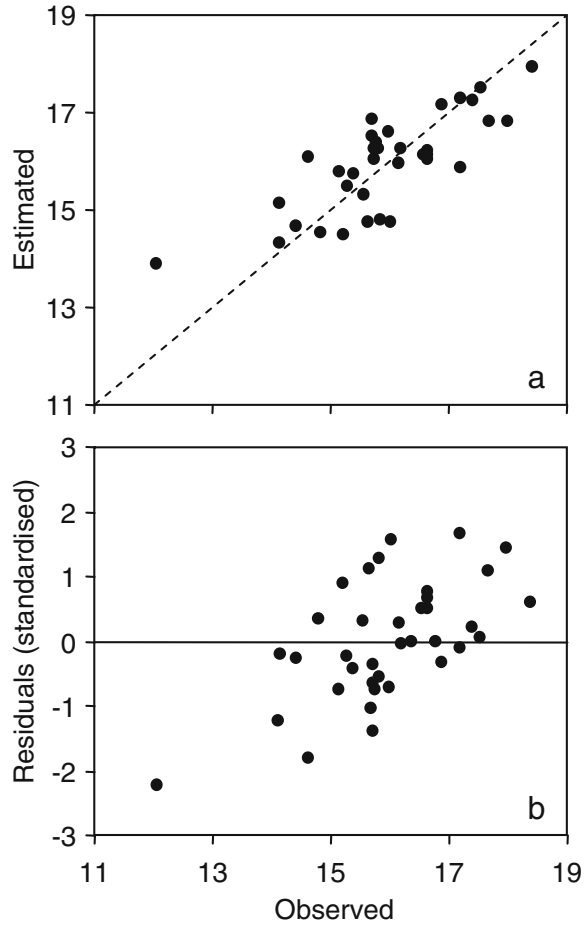

Fig. 5 Plot of estimated versus observed production of *F. verticillioides* conidia (**a**) and corresponding standardised residuals (**b**). Conidia are expressed as the natural logarithm of counts on maize stalk pieces placed on the soil between rows of maize crops (see Fig. 4). Estimates are calculated by the regression equation of Table 3. The *dotted line* (in **a**) represents the coincidence of estimated and observed data

Available water in stalk pieces placed in the field in 2004 varied between 0.41 and 0.83. The highest values were measured after the rainfalls of 24 July (a_W=0.83) and 5 August (a_W=0.81), while the lowest was found in the sample collected on 16 August after a period with high VPD (Fig. 5). Significant correlation was found between a_W and total precipitations (r=0.65, P=0.04, n=10), average RH (r=0.72, P=0.019), average VPD (r=−0.71, P=0.021), number of moist days in the 3 days before sampling (r=0.85, P=0.002), precipitations in the 7 days preceding sampling (r=0.85, P=0.002), but not with air temperature.

Discussion

The results of the present work generated new knowledge about the epidemiology of *F. verticillioides*, the causal agent of maize ear rot, in particular regarding the production of conidia on maize stalk residues. Comparison with the literature is unfortunately affected by the uncertainty arising from the revision of the species previously named *F. moniliforme*, which prevents the assumption of a complete equivalence between this name and *F. verticillioides*, particularly when dealing with older literature (Seifert et al. 2003).

The effect of temperature on the dynamics of spore production was in substantial agreement with previous studies (Jaurihar and Mehta 1973; Devi and Singh 1994; Tonapi et al. 2007) and confirmed the ability of the fungus to develop under a wide range of temperatures (Marín et al. 1995, 1996, 1999). Spore production occurred between 5°C and 45°C. Based on the nonlinear regression model fitted to the data, the optimum temperature was estimated to be 27°C. Differences between three temperatures around the optimum (25°C, 30°C and 35°C) were consistent over 18 fungal strains from different geographic origins showing varying sporulation capabilities. Range and amount of sporulation found by Melcion et al. (1997) in different strains were similar to those from this study.

Temperature must be considered as an environmental factor that influences spore production under field conditions, in addition to the availability of water (Hsieh et al. 1979), humidity (Indira and Muthusubramanian 2004; Tonapi et al. 2007), and alternate light and dark (Devi and Singh 1994).

Under the environmental conditions occurring after maize silk emergence at the experimental location in the west of the Po Valley (northern Italy), in 2003 to 2005, sporulation of *F. verticillioides* on stalk residues placed in a maize crop was continuous. In the 3 years conditions differed greatly: 2003 was particularly dry and hot, while 2005 was cooler with abundant and regularly distributed precipitation.

Variations in spore production were observed between and within years, ranging from 1.7×10^5 to 9.8×10^7 conidia g^{-1} of stalk. A multiple regression model was developed, accounting for 62% of the total observed variability, and showed that irrespective of weather conditions spore production on the stalk residues declined with time. This effect can be explained with the temporal changes in the chemical composition of the stalks, both in terms of carbon sources and mineral composition of the substrate (Deshpande 1969; Jaurihar and Mehta 1973; Hsieh et al. 1979; Shahid-Ahamad et al. 2004), which may change over time due to fungal feeding and the progressive degradation of the residues (Vanlauwe et al. 1994). Another possible cause is progressive colonisation of the residues by soil mycoflora that contain fungi able to suppress sporulation of *F. verticillioides* (Luongo et al. 2004; Sobowale et al. 2005).

The regression model also showed that the total amount of rain (or overhead irrigation) in the 2 weeks before sampling reduced the amount of conidia produced on the stalk residues, probably caused by the dispersal and wash-off of conidia (Ooka and Kommedahl 1977), as confirmed by the differences between stalks exposed and sheltered from rainfall in 2003.

The number of moist days in the previous 2 weeks had positive effects on spore numbers on stalks. It likely that this results from the favourable effect of high relative humidity (Tonapi et al. 2007) and of water availability in the substrate (Hsieh et al. 1979) on spore production. In fact, rain, irrigation and the flux from the atmosphere (measured by the VPD) all provided soil and the stalk residues with water. This was also confirmed by a_W in the stalk residues in 2004.

The degree-days of the previous three days also had positive effects on spore production. This results probably from the effect of temperature, which was between 17°C and 31°C, an interval where sporulation increases almost linearly with temperature, as found in the controlled environment experiments. Summer temperatures in this maize-growing area are usually within the above interval. This effect would probably have varied if experiments were done in hotter climates.

The present regression model was in substantial agreement with previous studies (Indira and Muthusubramanian 2004; de la Campa et al. 2005). de la Campa et al. (2005) developed a regression equation explaining 67% of the fumonisin found in maize kernels. Some weather variables in the 4 to 10 days before silking were included in the model as an estimate of the inoculum abundance. Temperatures <15°C and >34°C reduced, and rain increased, the production of fumonisin. Indira and Muthusubramanian (2004) found that spore production by some fungi affecting sorghum in India, including *F. moniliforme*, was positively correlated with relative humidity, the

amount of rainfall and the number of consecutive rainy days, but negatively correlated with temperature. Rainfall and relative humidity should provide sufficient moisture which is accounted for by the number of moist days in our equation. The negative effect of temperature observed in India could be due to high temperatures.

The results obtained in controlled experiments and in the field led to the conclusion that production of conidia by *F. verticillioides* occurs over a wide range of environmental conditions. In the 3 years considered here, the weather was very different, hot and dry in 2003 and very rainy in 2005; nevertheless, conidia were produced in high numbers on the maize stalk residues in the 30 days following silk emergence, which is the period of infection occurrence (Reid et al. 2002; Stewart et al. 2002).

Therefore, it can be concluded that abundant inoculum is present in the maize crops on residues colonised by *F. verticillioides* over different environmental conditions. Temperatures between 17°C and 27°C and moisture in the residues are particularly conducive.

These results stress the importance of managing maize crop residues to reduce the inoculum potential (Cotten and Munkvold 1998), especially in areas where maize is grown in short rotation or as a continuous crop, and provide information for the development of a simulation model for the life-cycle of *F. verticillioides* (Battilani et al. 2003).

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