EFFECT OF CONSTANT AND FLUCTUATING TEMPERATURE REGIMES ON SPORULATION OF FOUR FUNGI CAUSING HEAD BLIGHT OF WHEAT

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SUMMARY

Macroconidial production of four fungi causing head blight of wheat (*Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, *Microdochium nivale*) was studied on PDA under constant temperature regimes. Three isolates per species were used, which represented three groups of isolates showing different sporulation capability. Relationships between temperature (5 to 35°C), incubation time (1 to 21 days) and sporulation rate were analysed by a non-linear regression model. Differences between isolates within species did not modify the pattern of sporulation rate in relation to changing temperature. Based on this model, different responses of fungal species to temperature regimes were obtained, in terms of adaptability to different temperatures, speed in beginning production of spores and in reaching maximum sporulation. Considering these features, *F. avenaceum* was the highest performing fungus, followed by *M. nivale*, and then by *F. graminearum* and *F. culmorum.* The optimum temperature for macroconidial production was 32°C for *F. culmorum* and *F. gramineaum*, 28°C for *F. avenaceum*, and 26°C for *M. nivale*. In further experiments, a temperature-dependent model elaborated from the regression equations produced accurate estimates of the numbers of macroconidia produced on wheat stems which had been artificially infected with each of the four fungal species and placed onto the soil of a wheat crop. Under such natural conditions, temperature affected sporulation more than the other meteorological factors.

Key words: *Fusarium* species, *Microdochium nivale*, macroconidial production, temperature-dependent model.

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INTRODUCTION

Fusarium head blight (FHB), a complex disease caused by several fungi (Wiese, 1977), can be a serious disease affecting wheat and other cereals. This disease has the ability to affect yield severely when frequent rainfall, high humidity, and persistent dew coincide with the flowering and early kernel-fill period (Parry *et al.*, 1995). Damage caused by FHB is multifold and has major economic and social impacts, which make it urgent to find solutions for managing the disease (Bai and Shaner, 1994; McMullen *et al.*, 1997). The close dependence of this disease on the meteorological conditions occurring during a short period of the wheat-growing season suggests that a forecasting system for FHB epidemics should be elaborated (Parry *et al.*, 1995), supplying growers with information useful for effective use of fungicides and other disease management strategies (McMullen *et al.*, 1997).

Parry *et al.* (1995) showed that the production of inoculum on sources is the first aspect to be considered in elaborating such a model. Measurement of inoculum available on sources may provide an indication of the risk of severe epidemics if the weather is subsequently favourable for both inoculum dispersal and infection (Fitt *et al.*, 1989). For example, the number of *Septoria* spores on dead leaves at the base of winter wheat crops in the spring may provide an indication of the risk of subsequent epidemic development on the upper leaves (Royle *et al.*, 1986). Understanding the dynamic of spore production by fungi causing FHB has received little attention, so information is still incomplete (Parry *et al.*, 1995; McMullen *et al.*, 1997).

It is well known that *Fusarium* species all use infected host debris for carryover in soil (Atanasoff, 1920), where they survive saprophytically (Parry *et al.*, 1994). This debris may serve as a food base for sporulation (Cook, 1981) and is considered to be the principal reservoir of inoculum for the infection of wheat heads (Sutton, 1982). Inoculum for the development of a FHB epidemic can also arise from *Fusarium* infection on basal wheat stems (Parry *et al.*, 1995). Surveys on the incidence of common foot rot in wheat showed that this

disease is widespread and consistent; in northern Italy, the disease was present in 100% of crops assessed between 1988 and 1990, with high frequency of *M. nivale*, *F. avenaceum*, *F. graminearum*, and *F. culmorum* (Rossi *et al.*, 1995).

Production of spores occurs on infected host part or infested crop refuse on the ground where light is available (Cook, 1981). With *F. graminearum* sporulation is as common sexually (perithecia) as asexually (sporodochia) (Khonga and Sutton, 1988); perithecia may even serve as survival structure in the soil (Wearing and Burgess, 1978). For *F. graminearum*, foot rot is probably a less important inoculum source than head debris returned to the soil surface during harvest, due to different life cycles and the ecological requirements of group I and group II populations, causing foot rot and head scab, respectively (Francis and Burgess, 1977). *M. nivale* is also able to produce both perithecia and sporodochia on infected plant parts, which are responsible for aerial spread from plant to plant during the season (Cook and Bruehl, 1968; Millar and Colhoum, 1969); perithecia that overwinter on residues are usually empty in the following spring (Cook and Bruehl, 1968). With *F. avenaceum*, inoculum production is almost entirely asexual, although the perithecial stage has been seen (Cook, 1967). With *F. culmorum*, sporulation on infected host tissue is entirely asexual so far as it is known (Cook, 1968; Piglionica *et al.*, 1975).

Both ascospores and conidia are inocula for FHB infection (Millar and Colhoun, 1969; Stack, 1989); factors affecting production of ascospores by *Gibberella zeae* (teleomorph of *F. graminearum*) (Tschanz *et al.*, 1975; Tschanz *et al.*, 1976; Ye, 1980) and by *Monographella* (=*Calonectria*) *nivalis* [teleomorph of *F.* (=*Microdochium*) *nivale*] (Millar and Colhoun, 1969) have been studied, while the environmental conditions favouring production of conidia have not been investigated enough.

The present work was carried out within a framework of research aimed at developing a risk model for FHB of wheat. In detail, the relationships between environmental conditions and macroconidial production in four fungi causing the disease were considered. In the first phase of research, conidial production was studied under constant temperature regimes and functions relating sporulation with temperature and incubation time were developed; in the second phase, numbers of macroconidia produced under natural fluctuating temperatures were compared with those expected based on functions developed under controlled-environment experiments.

MATERIALS AND METHODS

Collection of fungi. To obtain isolates of the prevailing fungi infecting the wheat kernels produced in northern Italy, samples of kernels from several wheat-growing areas were collected starting from the early ’90s. Kernels showing discoloration or shrivelling were washed in running tap water for 20 min, sterilised with ethyl alcohol (70%) for 15 sec and then in sodium hypochlorite (2%) for 2.5 min, rinsed 3 times in sterile water and dried on absorbent paper under an air flow. Kernels were placed in Petri plates on water agar (1.2%) adjusted to pH 5.2. After 6 to 8 days of incubation at 25°C, the fungal colonies growing from the kernels were transferred to other plates on potato dextrose agar (PDA) with streptomycin sulphate (50 mg l^{-1}) and incubated at different temperatures (15 to 25°C), with 12 hours of light. *Fusarium* species were then identified according to Nelson *et al.* (1983) and Burgess *et al.* (1988). When necessary, isolates were transferred on to carnation-leaf agar, as described by the authors previously cited. Single-spore strains were obtained from the species identified and preserved.

In a preliminary work (unpublished), the isolates of *F. avenaceum* (Corda ex Fr.) Sacc., *F. culmorum* (Smith) Sacc., *F. graminearum* Schwabe, and *Microdochium nivale* (Fr.) Ces. were characterized for both morphology and colour of colonies, and tested for their mycelium growth and sporulation capability on PDA, at two temperatures (15 and 25°C); based on these characters, isolates of each species were grouped in three homogeneous clusters, using the clustering procedure of SPSS (SPSS inc., Chicago, Michigan). A representative isolate for each group was used in the present study. Specialised institutes have confirmed their identification. These isolates has been also used in a previous work (Rossi *et al.*, 2001).

Influence of constant temperature regimes on sporulation. PDA ‘starter’ plates were inoculated with each fungal isolate from stock cultures and incubated for one week at 20°C. Plugs (0.5 cm^2 wide) were then extracted from the periphery of these colonies with a cork borer and transferred to the centre of a PDA ‘test’ plate (9 cm diameter). These inoculated plates were incubated in the dark, for either 6 days at 25°C (isolates of *F. culmorum* and *F. graminearum*) or 8 days at 20°C (isolates of *F. avenaceum* and *M. nivale*): at the end of this period, each colony had grown nearly to the edge of its plate, with no measurable sporulation. At this moment the inoculum plug was removed; each plate was separated into 8 equal sectors (about 8 cm^2 each) by plastic dividers and sealed with Parafilm. In aggregate,

288 test plates were prepared, 24 plates for each isolate; the plates were managed according to a 4 x 3 x 6 factorial experiment, with 4 replicates: 4 fungal species, 3 isolates per species, 6 temperature regimes (10, 15, 20, 25, 30, 35°C). Different temperature regimes were obtained by incubating the plates in growth chambers, with a photoperiod of 12 hours with fluorescent light of 1500 lux. After 1 and 3 days of incubation, and then at 3-day intervals until the end of incubation period (21 days), a sector was removed from each plate. The surface of the plate sector was scraped with a scalpel and homogeneously diluted in 10 ml of sterile water; this suspension was decanted through two layers of cheesecloth to remove hyphae and agar. The number of conidia in the suspension from each plate sector was counted using a cytometer; the total number of conidia per cm^2 was estimated based on the 10 ml volume of water used to harvest the conidia and the area of the plate sector.

An additional experiment was performed for *F. avenaceum* and *M. nivale*, by incubating plates at a constant temperature of 5°C.

Two kinds of statistical analyses were applied. To study the effect of fungal species, isolates and temperature regimes (10 to 35°C) on sporulation, a factorial analysis of variance (ANOVA) was performed on numbers of conidia produced after 21 days of incubation; numbers were previously ln-transformed [ln (x +1)], to make variances uniform. Fisher's protected LSD test ($P = 0.05$) was used to determine differences between means (Gilligan, 1996).

To study the dynamic of spore production as a function of temperature and time of incubation, a non-linear regression analysis was applied. The following regression equation was used:

$$SPOR = (\alpha \cdot Teq^{\beta} \cdot (1-Teq))^{\phi}/(1+\exp(\delta-\varphi \cdot t)) \qquad [1]$$

where: SPOR is the sporulation rate; Teq is the equivalent of temperature (*sensu* Analytis, 1980) as (T-Tmin)/(Tmax-Tmin) (where T is the experimental temperature regime, Tmin is 5°C and Tmax is 35°C); t is the incubation time (1, 3, 6, …, 21 days); α, β, ϕ, δ, and φ are the parameters. The parameters were calculated using the non-linear regression procedure of SPSS; Teq and t were the independent variables, while SPOR was the dependent one. SPOR was calculated from the experimental data, dividing the numbers of conidia yielded by each fungal isolate and by each species (average of three isolates) after each incubation time by the number yielded after 21 days of incubation; thus, SPOR ranged between zero and one. The goodness of fit was evaluated on the basis of the standard error of parameters, the R^2 statistic, and the distribution of residues versus predicted values.

Regression equations were used to produce contour plots. These are two-dimensional representations of the three-dimensional plots, the latter originated by plotting the response variable (on to the vertical axis Y) on the plane originated by the two independent variables (temperature X1 and incubation time X2). In other words, they are a view from the top of the three-dimensional graph, where the response variable Y is represented by some contour levels drawn on to the X1X2 plane; 6 contour levels were used, corresponding to 0.05, 0.1, 0.3, 0.6, 0.9, and 0.95 of the sporulation rate.

Dynamic of spore yield under natural fluctuating temperatures. Wheat stems were inoculated with the four *Fusarium* species to produce infected residues, following Khonga and Sutton (1988, modified). Dry stem pieces (about 3 cm long) were immersed in distilled water for 12 hours, drained and autoclaved twice (150°C for 20 min each time). Stem pieces were separated into 4 groups, and placed in flasks; each group was inoculated with a conidial suspension ($5x10^6$ conidia ml^{-1}) of one of the four species, and incubated at 20°C for 7 days, in the dark. For each fungal species, the conidial suspension was prepared by mixing equal amounts of conidia produced, as previously described, by the 3 isolates used in the previously described laboratory experiments. At the end of incubation, stem pieces were abundantly colonised by the inoculated fungi, but sporulation did not yet occur.

Inoculated stems (40 g) were placed in 17 x 32 cm fibreglass-mesh bags (5 mm mesh). Bags were randomly placed above the soil in a winter wheat crop, at a distance of about 60 cm to avoid significant splash-contamination between neighbouring bags (Jenkinson and Parry, 1994). The crop was grown at Fiorenzuola (North Italy) in 1998, with a plant density of about 600 heads m^{-2} of soil (40 cm between rows) and a plant height of about 80 cm. The crop was managed according to the common use, with no fungicide sprays. Electronic equipment was installed about 100 m from the wheat plot, to measure hourly values of air temperature, relative humidity, and rainfall (at 1.5 m above the soil level). Two sets of bags were prepared: the first set (72 bags) was placed onto the soil on April 17 until May 2, the second one (84 bags) between May 5 and 23.

The stems were sampled when initially placed in the field and at 3-day intervals; at each sampling time, three bags were collected for each fungal species and stored at 4°C to be transported to the laboratory. The stems of each sample were weighed, and separated into two sub-samples. The first sub-sample was used to determine the moisture content of the wheat stems: stem pieces were weighed, dried at 50°C for 24 hours, finely crushed,

dried at 103°C for 4 hours, and then weighed; moisture content was then expressed in percentage by weight of water. Stem pieces of the second sub-sample were placed in flasks with 150 ml of distilled water and shaken vigorously for 30 sec. The macroconidia were then counted with a cytometer; the total number of conidia per g of stem (at 28% moisture) was estimated based on the volume of water used to harvest the conidia, the weight of the stem sample and its humidity level.

The data were used to compare numbers of macroconidia yielded under the fluctuating temperatures in the field with the dynamic of conidial production found in the experiments performed under constant temperature regimes. A variable SPOR' was created as the first derivative of equation [1], which represents the increase of SPOR in each time unit, as a function of temperature. Afterwards, a daily value of SPOR' was calculated using the daily mean temperature measured in the field; finally, these daily values were accumulated over the time of exposition of infected stems in the field, as follows:

$$\hat{SPOR}_i = \Sigma_{i=1}^{n} SPOR'_i \qquad [2]$$

where: i is the number of days of exposition (1 to 16 or 18, in the first and second exposition periods, respectively).

$\hat{SPOR}$ was then considered as an estimate of the sporulation under field conditions, based on the sporulation observed in environment-controlled conditions, and was compared with the observed conidial production. Pearson's coefficients of correlation were calculated as a measure of association between observed and estimated sporulation, in each fungus and stem exposition period.

RESULTS

Macroconidial production under constant temperature regimes. The ANOVA performed on the ln-transformed numbers of macroconidia yielded after 21 days of incubation under constant temperatures between 10 and 35°C showed that both species and isolates within each species significantly (at $P < 0.001$) affected sporulation. Also temperature regime significantly (at $P < 0.001$) affected sporulation, with significant interactions with fungal species and isolates (Fig. 1). High conidial production occurred at 30°C for all isolates of *F. avenaceum*; two isolates produced high spore numbers also at 25°C. For *F. culmorum* and *F. graminearum* maximum conidial production was always between 25 and 30°C. All isolates of *M. nivale* produced high numbers

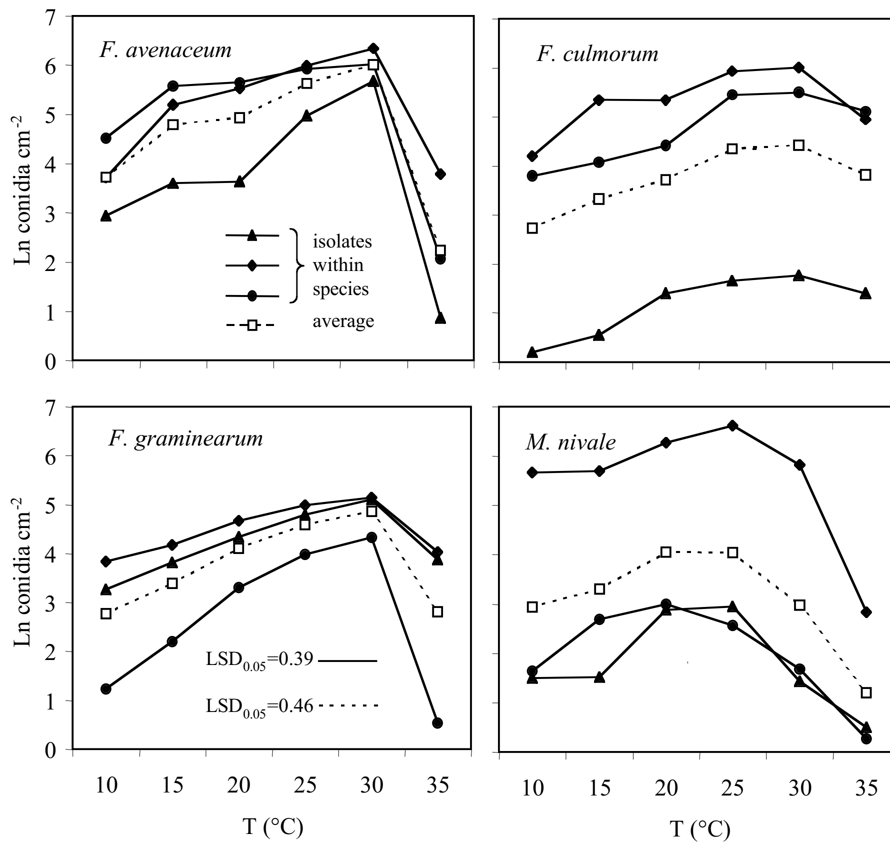

Fig. 1. Numbers of macroconidia produced (per cm^2 of fungal colony grown on PDA) by three isolates of four fungal species grown for 21 days at constant temperature regimes, between 10 and 35°C. Data are ln-transformed averages of each fungal strain (four replicates per strain) (—) and species (----), while LSD values are the correspondent least significant differences, at $P \leq 0.05$.

Table 1. Parameters and statistics of the regression equation [1] describing relationships between temperature (T = 5 to 35°C), incubation time (t = 1 to 21 days) and sporulation rate (SPOR = 0 to 1) of four fungal species (average of three isolates) grown on PDA at constant temperature regimes:
$SPOR = (\alpha \cdot Teq^{\beta} \cdot (1-Teq))^{\phi}/(1+\exp(\delta-\varphi \cdot t))$; $Teq = (T-5)/30$.

Parameter	*F. avenaceum*		*F. culmorum*		*F. graminearum*		*M. nivale*	
α	12.43	(3.446)[a]	20.92	(4.575)	25.98	(8.537)	6.64	(0.331)
β	4.27	(0.685)	8.12	(0.727)	8.59	(1.212)	1.99	(0.107)
ϕ	0.49	(0.118)	0.32	(0.038)	0.24	(0.047)	2.55	(0.406)
δ	1.78	(0.633)	7.83	(1.743)	5.52	(1.308)	4.31	(0.977)
φ	0.46	(0.173)	0.77	(0.180)	0.51	(0.131)	0.51	(0.121)
R^2	0.68[b]		0.88		0.80		0.86	

[a] Standard error of parameters.
[b] Coefficient of determination R^2.

of conidia at 20°C; two isolates produced similar numbers also at 25°C and one at 15°C. On average, *F. avenaceum* produced more macroconida than the other species at 10°C; at 35°C, *F. culmorum* produced the highest spore number, while *M. nivale* the lowest one (Fig. 1).

The relationships between temperature, incubation time and the sporulation rate of each isolate and species (mean of 3 isolates per species) were analysed by a non-linear regression procedure. When the equation [1] was applied to the four species, it produced a satisfactory fit of experimental data (Table 1): parameters had small standard errors, the coefficient of determination ranged between 0.68 (for *F. avenaceum*) and 0.88 (for *F. culmorum*), and residues were randomly distributed over the entire range of the independent variables (not shown). Similarly, a good fit was obtained when the equation [1] was applied to each fungal isolate (R^2 between 0.74 and 0.98). Estimates of parameters for each isolate of the same species were constantly within the standard errors of the parameters calculated for the average of such a species (Fig. 2). So, the differences between isolates within each species, though significant at the ANOVA, did not strongly differ for the rate of sporulation, both over incubation time and temperature. In other words, isolates of the same species were different for the numbers of conidia produced but not for the dynamics of spore production.

The contour plots resulting from the regression equations showed the different response of fungi to changing conditions for sporulation (Fig. 3). *F. avenaceum* showed the greatest adaptability to the different experimental conditions. The first contour level (0.05 SPOR) was drawn below 10°C, and a consistent macroconidial production had already occurred before 4 days of incubation; furthermore, this species was more rapid than the others in reaching the highest sporulation for each temperature regime. Maximum sporulation occurred at about 28°C. *F. culmorum* and *F. gramineaum* showed similar patterns of the contour levels in the range of temperatures considered, with very low sporulation before 8 days of incubation, irrespective of temperature. Both fungi showed a greater ability to produce macroconidia at about 32°C, but spore yield dropped steeply at higher temperatures. Sporulation of *M. nivale* occurred within a narrower temperature range compared to the other species. Maximum macroconidial production occurred at about 26°C; at the optimum temperature regime, this fungus was more rapid than *F. graminearum* and *F. culmorum* in beginning production of macroconidia, but it was slower in reaching the highest sporulation level.

Macroconidial production under natural fluctuating temperature. Macroconidial production on pieces of wheat stems, which had been artificially infected with each of the four fungi and placed on the soil of a wheat crop, occurred under changing meteorological conditions (Fig. 4). Mean daily temperature fluctuated between 8.5 and 19.3°C, and relative humidity between 51 and 92%. During the first exposition period, 17 mm rain fell on nine days, with a maximum amount of 6 mm on April 30, and a maximum intensity of 2.5 mm h^{-1} on April 7. In the second period, there were 6 rainy days, with a total rainfall of 2 mm; maximum intensity was 0.8 mm h^{-1} May 16. Moisture content of the wheat stem pieces at the time when initially placed in the field was 28%. In the first exposition period, moisture fluctuated between 27% and 33% until the last sampling time, when it increased to 51%; in the second period, moisture progressively decreased to 16-17% of the last three samples (Fig. 4).

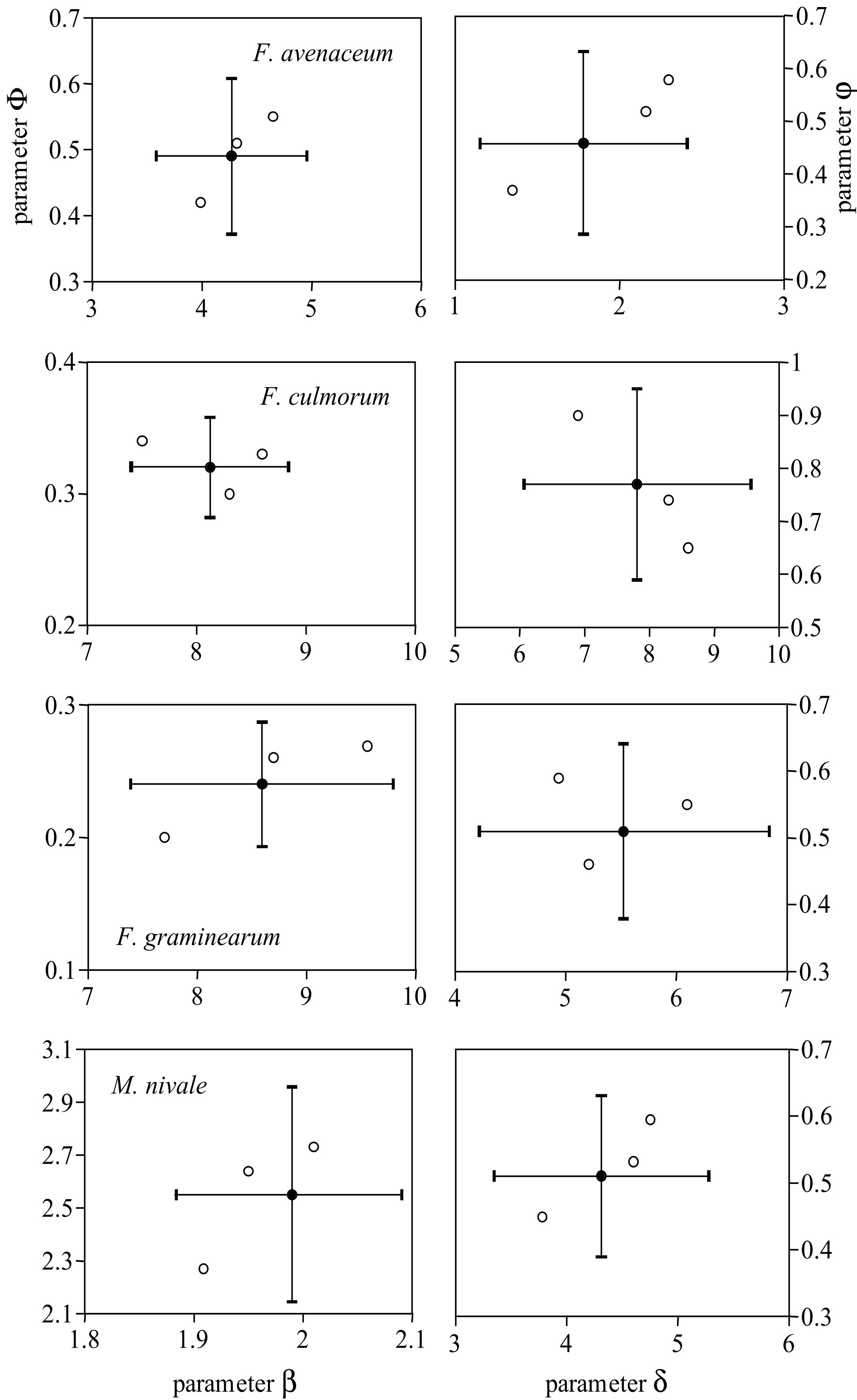

Fig. 2. Parameters of the regression equation [1] fitting the relationship between temperature (T, in °C), days of incubation and sporulation rate (on a 0 to 1 scale) of four fungi. Empty and filled points represent the parameters estimated for each isolate and for each species (average of three isolates), respectively; bars represent the variability (± standard error) of the parameters estimated for each species (see Table 1).

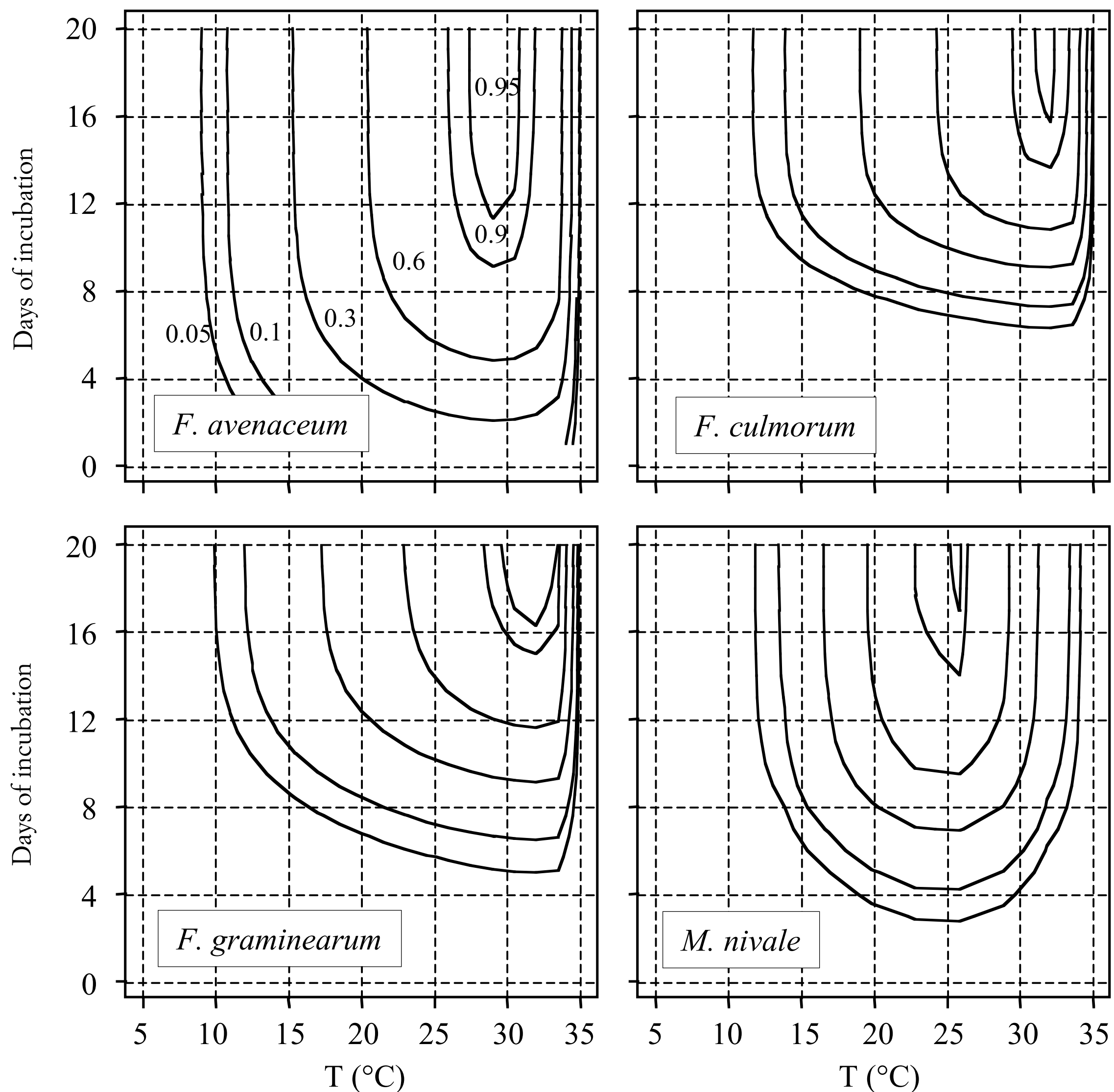

Fig. 3. Contour plot for the regression equation [1] fitting the data on the relationship between temperature (T, in °C), days of incubation and sporulation rate (on a 0 to 1 scale) of four fungi. Regression equations are shown in Table 1. The following contour lines were drawn: 0.05, 0.1, 0.3, 0.6, 0.9 and 0.95 sporulation rate.

The numbers of macroconidia produced under such natural conditions were significantly correlated with the sporulation rate observed during the experiments carried out under controlled-environment conditions (Fig. 4); coefficients of correlation between the number of macroconidia produced on wheat stems sampled at different times and the correspondent sporulation rate estimated according to equation [2] ranged between 0.91 and 0.99 (Table 2). Thus, the temperature-dependent model accounted for a very high part of variance, from 83% to 98%; the residual part of variance included the effect of all other factors influencing macroconidial production under field conditions. The greatest differences between observed and estimated sporulation occurred for stem samples collected on April 26 and 29 for *F. avenaceum*, on April 26 for *M. nivale*, on May 17 for *F. culmorum* and *F. graminearum* (Fig. 4); all these samples were collected after rain events: 2 mm rainfall on April 23; 1 mm on 24 to 26 April; 1.5 mm on 15 to 17 May.

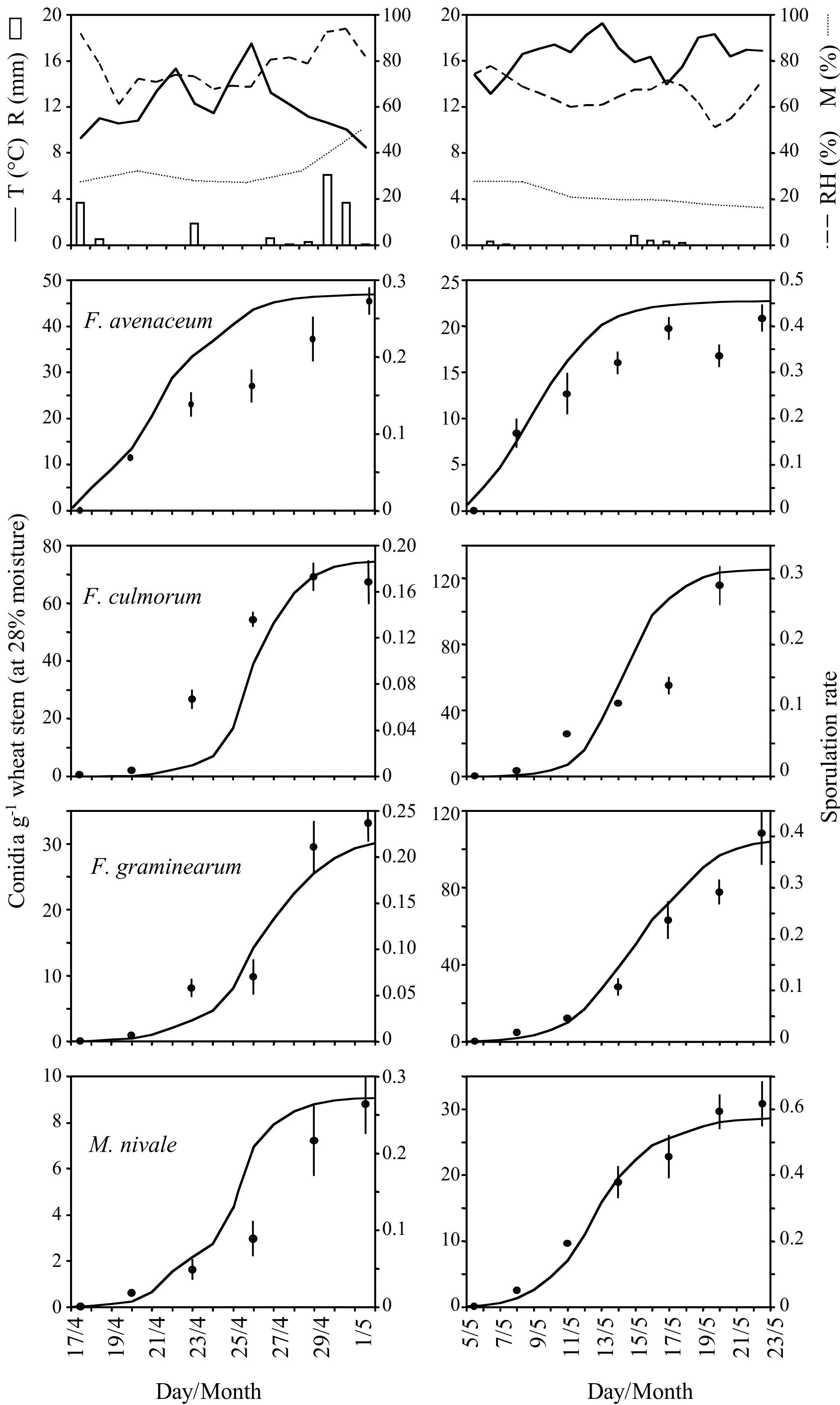

Fig. 4. Relationships between numbers of macroconidia (•) produced by four fungi, on wheat stems placed above the soil of a wheat crop under different conditions of temperature (T), relative humidity (RH), rainfall (R) and stem moisture content (M), and the sporulation rate (—) calculated by the temperature-dependent model [2]. Vertical bars extending from points are the standard errors of spore numbers.

Table 2. Coefficients of correlation between numbers of macroconidia produced on pieces of wheat stems artificially infected with four fungal species, exposed to natural conditions above the soil of a wheat crop during two periods, and sporulation estimated by the temperature-dependent model [2].

Fusarium species	First exposition period April 17 to May 2, 1998			Second exposition period May 5 to 23, 1998		
	r[a]	n[b]	P[c]	r	n	P
F. avenaceum	0.95	6	***	0.97	7	***
F. culmorum	0.95	6	***	0.91	6	**
F. graminearum	0.97	6	**	0.98	7	***
M. nivale	0.92	6	**	0.99	7	***

[a] Pearson's coefficient of correlation.
[b] Number of experimental data.
[c] Significance level:** significant at $P \leq 0.01$; *** significant at $P \leq 0.001$.

DISCUSSION

Fungal species were able to produce macroconidia at all the temperature tested, 5 to 35°C. This result was in agreement with Srobar (1978), who used the same species, with the exception of *M. nivale*, isolated from wheat, but was in contrast with Devi and Singh (1994), who did not observe sporulation on *F. graminearum* and *F. avenaceum* isolated from rice, in the intervals 5 to 15°C and 35 to 38°C. Andersen (1948) found that *F. graminearum* did not sporulate at 36°C; Millar and Colhoun (1969) found that *M. nivale* produced conidia over a temperature range of 8-22°C, but it was not indicated if higher temperatures were tested.

The dynamic of sporulation of each species in relation to temperature regime and incubation time was described by a specifically designed equation [1], which is a type of logistic model. The general form of the logistic model is written as: $y=c/(1+\exp(a-b\cdot x)$ (Campbell and Madden, 1990), which draws a sigmoid curve of y over x: the parameter c estimates the maximum level of y, a depends on the initial value of y, and b is a rate parameter. In the model used in this work, the parameter c was replaced by the function of Analytis (1980) which accounted for the shape of spore yield in response to increasing temperature, while the parameters δ and φ accounted for the S-shaped increase of spore yield over the incubation time. These parameters were estimated for each isolate and species, the latter as an average of the isolates belonging to the same species. Since it is well known that there is variability in the sporulation capability within the same fungal species (Srobarova and Bukovcakova, 1989; Kanatani and Takeda, 1991), isolates used in this work were selected as representative of three groups with different spore yield. Actually, isolates showed significant differences in the numbers of conidia produced. However, their patterns of macroconidial production in relation to changing temperatures did not change markedly, since the model parameters estimated for each isolate were within the range of variability of those estimated for the species. Therefore, the models calculating the average SPOR for each fungal species (Table 1) were considered representative of the dynamics of macroconidial production of such a species.

Based on these equations, different responses of fungal species to temperature regimes were outlined, in terms of adaptability to different temperatures, speed in beginning to produce spores and in reaching maximum sporulation. Considering these features, *F. avenaceum* was the highest performing fungus, followed by *M. nivale*, and then by *F. graminearum* and *F. culmorum.* The optimum temperature for macroconidial production was 32°C for *F. culmorum* and *F. gramineaum*, 28°C for *F. avenaceum*, and 26°C for *M. nivale*.

A critical comparison between these results and earlier works was not easy, because a few works have been published on this topic. Devi and Singh (1994) found that the optimum temperature for both *F. avenaceum* and *F. graminearum* was 30°C, compared to 25 and 35°C. Based on discrete temperature intervals, this result was in agreement with that obtained in this work; nevertheless, using the regression equations a difference of about 4°C between these two species was found, maximum sporulation being at 28°C for the former fungus and at 32°C for the latter one, in complete agreement with Andersen (1948). Precise data on the other fungal species were not available; however, there was a substantial agreement between the temperature relationships found in this work and the temperature re-

quirements of different species inferred from their distribution: *F. graminearum* is present in the warmer regions, *F. culmorum* is intermediate in temperature preferences, *F. avenaceum* prefers cool climates, while *M. nivale* is widespread in cooler climates (Cook, 1981; CABI, 2000).

The regression equations were used to elaborate a temperature-dependent model estimating sporulation on wheat stems placed on the soil surface in a wheat crop, under changing meteorological conditions. Changes in the numbers of macroconidia measured between successive wheat stem samples depended on the quantity of new spores produced minus the quantity removed during the intervening period (Fitt *et al.*, 1989). As shown in this work, temperature is an important factor involved in macroconidial production; the moisture of wheat stems must be considered important too (Sung and Cook, 1981). Rainfall is the most important factor for either the splash removal of macroconidia produced on sporodochia (Stepanov, 1935; Jenkinson and Parry, 1994) or the spore loss through washing-off.

Since the model produced accurate estimates of actual data and accounted for a high part of experimental variance, it can be assumed that temperature affected the numbers of macroconidia produced on wheat stems more than the other cited factors. The effect of rainfall was unclear. In some cases, fewer conidia than expected were found in the stem samples collected after rain events; thus, removal of macroconidia due to the rain drops impacting on the sporulating stems could be presumed. However, in other cases, more abundant rainfall did not produce the same effect. In particular, stems sampled on May 2 were collected after 5 rainy days, with intense rainfall the day before, but the numbers of macroconida produced on the stems did not show any significant reduction compared to the expected ones. Thus, the effect of rainfall on macroconidial removal from inoculum sources in basal wheat crops should be further investigated. However, results demonstrated that macroconidia of the four fungal species are present on infected wheat residues over a wide range of environmental conditions, and that neither rainfall nor wetting of the inoculum sources hindered macroconidial production. Therefore, the temperature-dependent model might be useful in predicting in the field the risk of a severe FHB epidemic will occur if weather conditions are favourable for spore dispersal and infection.

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