

INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE INFECTION OF WHEAT SPIKES BY SOME FUNGI CAUSING FUSARIUM HEAD BLIGHT

V. Rossi, A. Ravanetti, E. Patteri and S. Giosuè

Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, I-29100 Piacenza, Italy

SUMMARY

The effect of temperature and humidity on the infection of wheat head tissues by four fungal species causing head blight (*Fusarium avenaceum*, *F. graminearum*, *F. culmorum*, *Microdochium nivale*) was studied by controlled-environment experiments. Detached spikes were inoculated at flowering with a spore suspension and incubated at different temperatures (10.0 to 35.0°C), wet and in a saturated atmosphere. Infection frequency of glumes after different incubation times (4 to 72 hours) was determined by re-isolation of the fungi that had been inoculated. Regression models were then elaborated and used to compare species and to define their cardinal temperatures for infection. *F. avenaceum* and *F. graminearum* showed the highest infection frequency, with optimum at 28.0-29.0°C; *M. nivale* and especially *F. culmorum* had a lower infection frequency, with optimum at 18.0°C and 26.5°C, respectively. Spikes were then inoculated at optimum temperature for each species, either in the presence or in the absence of a film of water, under different regimes of relative humidity (100% to 65%). Reduction of relative humidity during incubation increased the frequency of glumes infected by *F. culmorum*, whereas it created unfavourable conditions for the other species.

Key words: wheat scab, *Fusarium* species, head infection, environmental conditions, regression models.

INTRODUCCION

Fusarium head blight (FHB) is a serious disease of wheat in many areas of the world (Parry *et al.*, 1995). Up to 17 causal organisms have been associated with the disease, but the most common species are *Fusarium graminearum* Schwabe, *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W.G. Smith) Sacc., *F. poae* (Peck) Wollenw.,

and *Microdochium nivale* (Fr.) Samuels *et Hallet* (Wiese, 1977; Parry *et al.*, 1995). Damage caused by the disease is multifold: reduced yields, discoloured and shrivelled kernels, which may be light enough to be expelled from the combine with the chaff during harvest, reduced test weight of harvested grain, lowered market grade (Bechtel *et al.*, 1985), contamination with mycotoxins (Bottalico *et al.*, 1989), reduction in seed quality due to reduced germination, seedling blight and poor stand (Tuite *et al.*, 1990).

Though FHB is a potentially destructive disease, its severity varies greatly in different years and locations, being strictly dependent on the epidemiological conditions; in many wheat-growing areas the occurrence of destructive outbreaks is often sporadic. Frequent rainfall, high humidity, and heavy dew that coincide with the period of crop susceptibility, which extends from flowering to the soft dough stage of kernel development (Pugh *et al.*, 1933; Andersen, 1948), favour infection. Under these conditions, spores germinate and the germ tubes grow into head tissues. In addition to weather, other factors contribute to the development of FHB epidemics, including high proportions of minimum tillage, high percentage of surfaces planted with host crops, and short rotation intervals between susceptible crops, which all favour the survival of the causal fungi on the residues of host crops and increase the amount of inoculum (McMullen *et al.*, 1997).

Control measures are mainly based on sanitation, since efficacy of aerial application of fungicides is often of doubtful effect. Problems associated with timing of fungicide application are among the main reasons for the variable efficacy of sprays (Parry *et al.*, 1995). A system forecasting the time of infection establishment could be a useful tool in improving the efficacy of fungicide application. Given the dependence of FHB epidemics on a wet and warm growing season, and the relatively short period of susceptibility of heads to infection, it would appear that FHB is a possible disease for forecasting, but Parry *et al.* (1995) stressed the need for further epidemiological studies. In particular, environmental conditions favourable to the infection were not investigated exhaustively. In fact, some reports

Corresponding author: V. Rossi
Fax: +39.0523.599256
E-mail: PATOLO@PC.UNICATT.IT

showed the dependence of *F. graminearum* on temperature and wetness (Atanasoff, 1920; Dickson *et al.*, 1921; Pugh *et al.*, 1933; Andersen, 1948; Bai and Shaner, 1991), but less information is available for other important fungal species causing FHB.

The objective of this study was to determine the effect of environmental conditions on the infection of wheat spikes by fungal species causing FHB, with reference to *F. avenaceum*, *F. culmorum*, *F. graminearum*, and *M. nivale*, which are the prevailing species infecting wheat in Italy (Rossi *et al.*, 1991; Orsi *et al.*, 1994; Porta-Puglia and Santorelli, 1997; Corazza *et al.*, 1997; Pancaldi and Torricelli, 1998). The main goal of this work was to obtain mathematical equations accurately fitting the relationships between temperature, humidity and infection, as a first step towards developing a forecasting system for the disease. For this reason, spikes were artificially inoculated with fungal inocula and incubated under different conditions of temperature and relative humidity, either wet or dry, and the resulting infection frequency of spike tissues was detected.

MATERIALS AND METHODS

Collection and maintenance of fungal isolates. To obtain isolates of the prevailing *Fusarium* species 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 a sterile air flow. Kernels were placed in Petri dishes on water agar (1.2%) adjusted to pH 5.2. After 6 to 8 days of incubation at 25.0°C, fungal colonies growing from the kernels were transferred to other Petri dishes on potato dextrose agar (PDA) with streptomycin sulphate (50 mg per l) and incubated at different temperatures (15.0 to 25.0°C), with an artificial day length of 12 hours. *Fusarium* species were then identified according to Nelson *et al.* (1983) and Burgess *et al.* (1988). When necessary, isolates were transferred onto carnation-leaf agar, as described by Nelson *et al.* (1983). Single-spore isolates were obtained from the species identified and preserved.

Three isolates of *F. avenaceum*, *F. culmorum*, *F. graminearum* and *M. nivale* were used in the present study. Specialised institutes have confirmed their identification. To produce conidia for artificial inoculations, these isolates were placed on PDA and processed as previously described.

Inoculation of wheat spikes. Spikes of bread wheat (*Triticum aestivum* L.) 'Centauro' were collected at one half anthesis completed (stage 65 of the Zadoks scale, Zadoks *et al.*, 1974), from a crop grown at Parma (North Italy) in 1997. The crop was grown according to the common use, following alfalfa, with no aerial fungicide application; environmental conditions before the collection of spikes were dry, so that natural *Fusarium* infection was supposed to be nearly absent. To better manage the experiments, detached spikes were stored in plastic bags at -18°C until their use; a preliminary experiment showed that this treatment did not significantly influence ability of the fungi to infect spike tissues. For inoculation, spikes were then maintained at 4°C for 24 hours, washed in running tap water per 20 min, sterilised with 2% sodium hypochlorite for 2 min and rinsed with sterile water. Spikes were then dipped in a spore suspension, continuously shaken. Spore suspension was prepared as follows: conidia produced by each fungal isolate on PDA were suspended in a solution of sterile water, streptomycin sulphate and neomycin sulphate (both at 50 mg per l), and Tween 20 (0.1%), until a concentration of $2 \cdot 10^4$ conidia ml⁻¹. The suspension used for inoculation was finally prepared by mixing equal amounts of the three suspensions produced for each strain of the same species. The previously mentioned solution, without conidia, was used to process the uninoculated test.

Influence of temperature on glume infection. The effect of temperature (T) on glume infection was studied by storing the inoculated spikes at constant T between 10.0 and 35.0°C, for different incubation times (t).

The inoculated spikes, still wet, were placed in aluminium boxes (30 x 20 cm) over a wire support, so that they did not lay on the box bottom. To keep the spikes wet, the bottom of the box was covered with wet blotting-paper, then the box was closed and put in a sealed plastic bag. The boxes were incubated at 10.0, 15.0, 20.0, 25.0, 30.0, and 35.0°C for 4, 8, 16, 24, 48, and 72 hours. For each treatment (fungal species x T x t) three replicates were prepared (*i.e.* three boxes), each containing three spikes. The experiment was repeated once.

At the end of each incubation time, 10 spikelets were randomly sampled from each spike. Then, lemma and palea of the central floret were collected, the latter glume including the ovary. Glumes were sterilised with sodium hypochlorite (0.5%) for 1 min, rinsed in sterile water and dried under an air flow. Lemma and palea were then placed separately in Petri dishes on agar amended with Dicloran (2 mg l⁻¹), Chloramphenicol (100 mg l⁻¹), streptomycin sulphate and neomycin sulphate (both at 50 mg l⁻¹), and incubated for 10 days at

20.0°C (for spikes inoculated with *M. nivale*) or at 30.0°C (for the ones inoculated with the other species); glumes from uninoculated spikes were incubated at both temperatures. At the end of this period, the fungal colonies grown from either lemma or palea were observed microscopically to identify the fungal species, as previously described. The frequency of infected glumes (lemma and palea) was then expressed as a percentage of the 30 glumes processed per replicate (10 lemma and 10 palea x 3 spikes).

Influence of humidity on glume infection. The effect of humidity on glume infection was studied by storing the inoculated spikes at constant T for 72 hours, at different regimes: wet, in a saturate atmosphere, or dry, at relative humidity (RH) between 95 and 65%.

Spikes were inoculated as previously described and incubated at 30.0°C (for *F. avenaceum*, *F. culmorum* and *F. graminearum*), at 20.0°C (for *M. nivale*), or at both temperatures (for the uninoculated spikes), inside the aluminium boxes, wet and in saturate atmosphere. After 24 hours of incubation, spikes were put inside beakers (volume of 1000 ml): spikes were inserted vertically in a specific support that allowed their basal culm (5 cm in length) to be dipped into a smaller beaker which contained 15 ml of Hoagland's nutrient solution (Hoagland and Arnon, 1950). The beakers were then closed with a plastic film and incubated again for 48 hours, with alternating light (12 hours dark/12 hours light), at the previously indicated temperatures, at RH of 100, 95, 85, 75, or 65%. The scheduled humidity regime inside the beaker was maintained as follows. In a first treatment, spikes were kept wet also after the first 24 hours of incubation, and 100% RH was obtained by pouring water on to the beaker bottom. In the other treatments, spikes were dried under an air flow after 24 hours of incubation, before being placed inside the beaker; the different RH regimes were obtained by pouring water into the beaker or by fixing a small gauze bag containing NaCl-salts to the internal wall of the beaker, as indicated in Table 1. Maintenance of the scheduled RH regime was continuously monitored by an electronic sensor placed inside the beaker. In every treatment, 5 ml of Hoagland solution were added after 24 hours and, where present, the salts were replaced. For each treatment (fungal species x humidity regime) three replicates were prepared (*i.e.* three beakers), each containing three spikes. The experiment was repeated once.

At end of the experiment, the frequency of glumes infected by the *Fusarium* species that had been inoculated was determined by re-isolation, as previously described. In this experiment, infection frequency was

calculated irrespective of the glume type.

Data analysis. An analysis of variance (ANOVA) was performed to determine the effect of treatments on the infection frequency of glumes, using SPSS (SPSS Inc., Chicago, Illinois, U.S.). Infection frequencies were transformed by the arcsine-square root calculation to correct nonnormality in residuals. A preliminary analysis of variance for the two repetitions of both experiments showed similar trends in *P* values of the F statistics for the effects of blocks, treatments and their interactions; moreover, the repetition x treatments interaction was not significant. Therefore the two repetitions of experiments were combined for further analysis. The full data set, including uninoculated control spikes, was preliminarily analysed. Since uninoculated spikes were nearly always infection-free, control data were excluded in the final analysis in order to avoid unequal variances between the inoculated and uninoculated treatments.

The two experiments were analysed separately, using a factorial design. In the first experiment, 4 factors were considered: *Fusarium* species (with 4 levels corresponding to the 4 species), temperature (with 7 levels, 10.0°C to 35.0°C), incubation time (with 6 levels, 4 to 72 h), and glumes (with two levels, palea and lemma). In the second experiment, 2 factors were included: species (with the 4 levels) and conditions of humidity (with 5 levels, 100% to 65% RH). The 3 spikes inside each box (first experiment) or beaker (second experiment) were considered as an experimental unit. The Fisher's protected LSD test (*P* = 0.05) was used to determine differences between treatments.

The quantitative relationships between the infection frequency of glumes, temperature and incubation time were analysed using multiple regression; the infection frequency of glumes was transformed into $\text{Ln}(Y_i+1)$ and used as response variable, whereas temperature (T) and incubation time (t) were used as regressors, alone or combined (T, t, T²...T⁴, t², t³, T·t, T²·t ... T⁴·t, T·t², T·t³, T²·t²). In this analysis the average infection frequency of the two glume types was used to produce equations explaining the ability of each fungus to infect head tissues under the different experimental conditions. These equations were considered more useful for a practical use than separate equations for lemma and palea. For each fungus, the best regression model was selected using a stepwise variable selection procedure; the procedure operated backward beginning with a model containing all the variables and removing them one at a time according to the significance of their contribution, based on an 'F to remove' criterion (F = 4). Models were evaluated on the basis of the standard error of model parameters, the residual distribution, the

determination coefficient (R^2 , which represents the proportion of variance of the response variable that is predictable from the regressors), the adjusted R^2 (R^2_{adj} , that adjusts for the number of independent variables in the model), the R^2 calculated for the back-transformed data (R^2_{back}). The last statistic was calculated as the determination coefficient of the simple regression between the observed values of infection frequency Y_i and the data obtained by back-transforming the model estimates, from $\text{Ln}(\bar{Y}_i+1)$ to \bar{Y}_i , for any X_{n_i} .

Regression equations were used to produce contour plots. They 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 plain originated by the two independent variables (temperature X_1 and incubation time X_2). 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 X_1X_2 plain; 7 contour levels were used, corresponding to 0.1, 1, 3, 10, 30, 60, and 90% of infection frequency.

Regression equations were also used to calculate the cardinal temperatures (minimum, optimal, and maximum) for the infection by each fungus; to calculate minimum and maximum temperatures the regression equations were solved by giving $t = 72$ and $Y = 0$; to calculate the optimal temperature, the first derivative of equations were solved by giving $t = 72$ and $Y' = 0$.

RESULTS

Influence of temperature. In the first experiment, spikes were inoculated with the four fungi and incubated at different temperatures, wet and in a saturated atmosphere. Infection frequency of glumes showed high variability, due to all the experimental variables considered: the ANOVA showed that all the factors (fungus, temperature, incubation time, and glume type), and their interactions, significantly ($P < 0.01$) influenced the response variable: the fungus accounted for 4% of total variance, the temperature for 13%, interaction fungus x temperature for 7%, incubation time for 17%, and interactions of incubation time x fungus and x temperature for 41% in aggregate. The residual variance was attributed to the differences between palea and lemma (3%), to their interactions with the other variables (9%), and to the experimental error (6%).

Interaction showed that the fungus, the incubation time and the type of glume were not independent in determining the infection frequency: thus, the effect of a factor was dependent on the selected level for the others. Nevertheless, the main effect due to the fungal

species, and the lower order of interaction resulting from the confounding of the glume type, deserved an independent comment. In fact, since the different combinations of influencing factors included in the experiment are a representative set of possible environmental conditions occurring during natural infections, the average effect of the fungal species produces information on the different infection ability of the four fungi under such conditions. Besides, differences between lemma and palea are due to their position inside the spikelets, being respectively in the outer and inner part; thus, their confounding produces a measure of the ability of each fungus to infect head tissues under the different experimental conditions.

On the average, *F. graminearum* and *F. avenaceum* showed the highest levels of infection frequency, followed at a significant lower level by *M. nivale*, and then by *F. culmorum*. Because of a significant ($P < 0.01$) interaction between fungal species and incubation time, differences between fungi were not significant at 10.0, 15.0 and 35.0°C, while they were highly significant at 20.0 to 30.0°C (Fig. 1). The maximum level of glume infection was 72.2% for *F. graminearum* and 62.8% for *F. avenaceum* (both expressed as an average of infection frequency of lemma and palea, after 72 hours incubation at 30.0°C), 11.7% for *M. nivale* (after 72 hours at 15.0°C), and 5.6% for *F. culmorum* (after 72 hours at 25.0°C) (Fig. 1).

The lowest order of interaction was also significant ($P < 0.01$), so that for any fungus and incubation time the infection frequency depended from the glume type. An example of this interaction was shown in Fig. 2 for a selected value of incubation temperature. For *F. avenaceum* and *F. graminearum* (at 30.0°C), no significant infection occurred on the outer glume (lemma) until 24 hours incubation had passed, whereas at least 48 hours were necessary to produce infection on the inner glume (palea); for *M. nivale* (at 15.0°C) and *F. culmorum* (at 25.0°C) the minimum time to produce infection on lemma was 48 and 72 hours, respectively, whereas a very few infections took place on palea even after 72 hours incubation. After the previously cited minimum times required for infection, infection frequency increased over incubation time following different patterns for the four fungi; in any case, infection was more frequent on lemma than on palea, but the increase of infection frequency followed similar patterns on the two glume types.

The regression analysis confirmed a close dependence of the infection frequency of glumes on the interaction between temperature and incubation. In fact, linear combinations of the two independent variables were always selected as significant (at $P < 0.01$) variables in

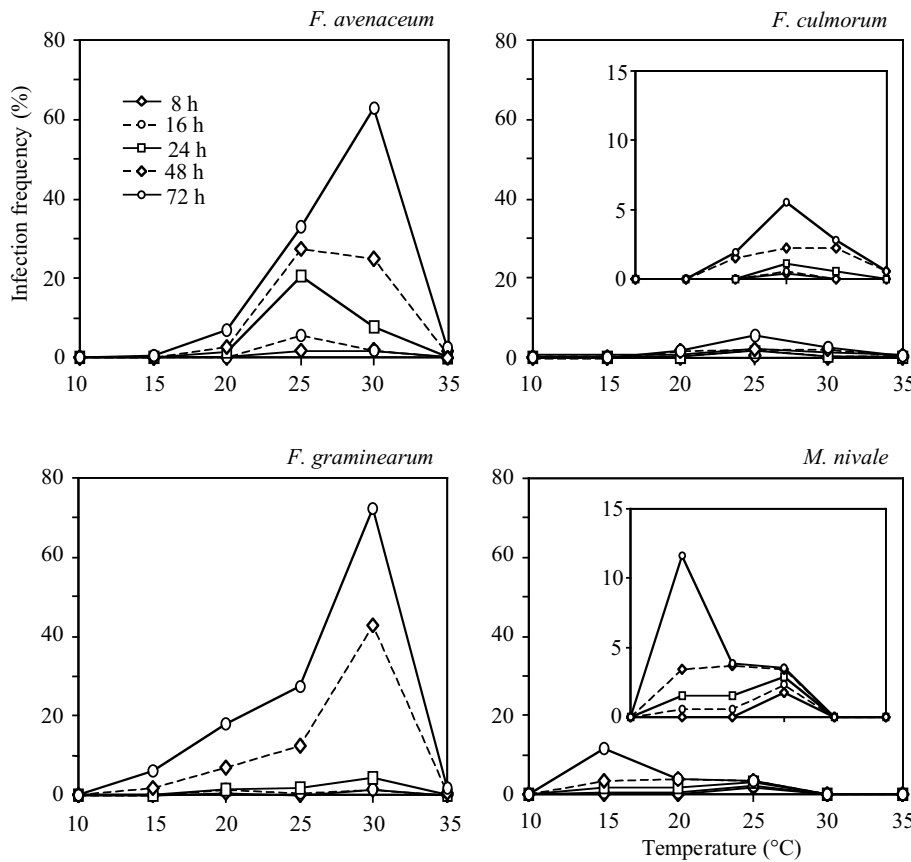


Fig. 1. Frequency of glumes (palea and lemma) infected by four fungal species causing head blight of wheat, after different times of incubation at different temperatures. The ANOVA performed on the arcsin-square root transformed data showed the significant ($F_{90,672} = 25.16$) effect of the interaction 'fungus x temperature x incubation time', with $LSD_{0.05} = 3.33$. Data in the graph are true percentages. Data obtained at 0 and 4 h incubation are not shown because they were always equal to 0% of infected glumes. In this graph the effect of glume type was confounded to show infection ability of the four fungi on the whole spike tissue.

Table 1. Experimental conditions used to incubate wheat spikes inoculated with *Fusarium* species causing head blight under different conditions of humidity.

Temperature of incubation (°C)	Relative humidity measured inside the beaker ^a (%)	Water ^b (ml)	NaCl ^c (g)
30	100 ^d	600	–
	95 ± 2	600	–
	85 ± 2	–	–
	75 ± 2	–	10
	65 ± 2	–	25
20	100 ^d	600	–
	95 ± 2	–	–
	85 ± 2	–	10
	75 ± 2	–	50
	65 ± 2	–	65

^a The beaker had 1000 ml of capacity and contained a smaller beaker containing 15 ml of a Hoagland solution.

^b Water covered the bottom of the beaker.

^c NaCl was placed inside a gauze bag fixed to the internal wall of the beaker.

^d Spikes of this treatment were putted into the beaker wetted by a film of water, whereas in the other treatments spikes were previously dried.

to the regression equations (Table 2); incubation was always included raised to the first power, to fit the exponential trend of the response variable over the time after inoculation, whereas temperature was included in combination with incubation time, raised to different powers, to fit the curvilinear response of infection frequency to changing temperatures. Different powers were included for the different fungi because they had a different response to the increasing temperature: for instance, *F. avenaceum* and *F. graminearum* had about the same infection frequency after 72 hours incubation at 25.0 to 35.0°C, but the former had lower frequencies at 15.0 and 20.0°C (Fig. 1). The intercept of the regression equations was always not significantly different from zero, to testify the absence of infection at the beginning of the experiment (time = 0); on the contrary, estimates of the other model coefficients were significantly different from zero and had low standard errors. Statistics showed that the regression equations fit the experimental data satisfactorily (Table 2). The worst fittings were obtained for *M. nivale* and *F. culmorum*, with R^2_{adj} equal to 0.64 and 0.69, respectively, but the R^2 for the back-transformed estimates showed that, in both cases, the estimates were sufficiently accurate compared to the actual data.

Table 2. Parameters and statistics of the regression model used to fit the relationship between infection frequency of glumes (%), temperature (T in °C) and incubation time (t in hours after inoculation).

Fungal species	Regression models				R ²	R ² _{adj} ^a	R ² _{back} ^b
<i>F. avenaceum</i>	0.137 - 0.00042·T ² ·t (0.00061) ^c	+ 0.000041·T ³ ·t (0.000004)	- 0.0000000828·T ⁴ ·t (0.000000008)		0.89	0.88	0.86
<i>F. culmorum</i>	- 0.009 + 0.09049·t (0.02971)	- 0.01746·T·t (0.00465)	+ 0.00101·T ² ·t (0.00022)	- 0.000017·T ³ ·t (0.000003)	0.72	0.69	0.84
<i>F. graminearum</i>	- 0.099 - 0.363·t (0.096)	+ 0.07808·T·t (0.02061)	- 0.00591·T ² ·t (0.00153)	+ 0.000199·T ³ ·t - 0.0000024·T ⁴ ·t (0.000048) (0.0000005)	0.95	0.94	0.87
<i>M. nivale</i>	0.89 - 0.1703·t (0.0413)	+ 0.02649·T·t (0.00646)	- 0.00109·T ² ·t (0.00031)	+ 0.000013·T ³ ·t (0.000005)	0.67	0.64	0.69

^a Coefficient of determination adjusted for number of parameters.

^b Coefficient of determination for the regression between observed data and back-transformed estimates.

^c Standard errors of model parameters; intercept is always not significantly different from zero.

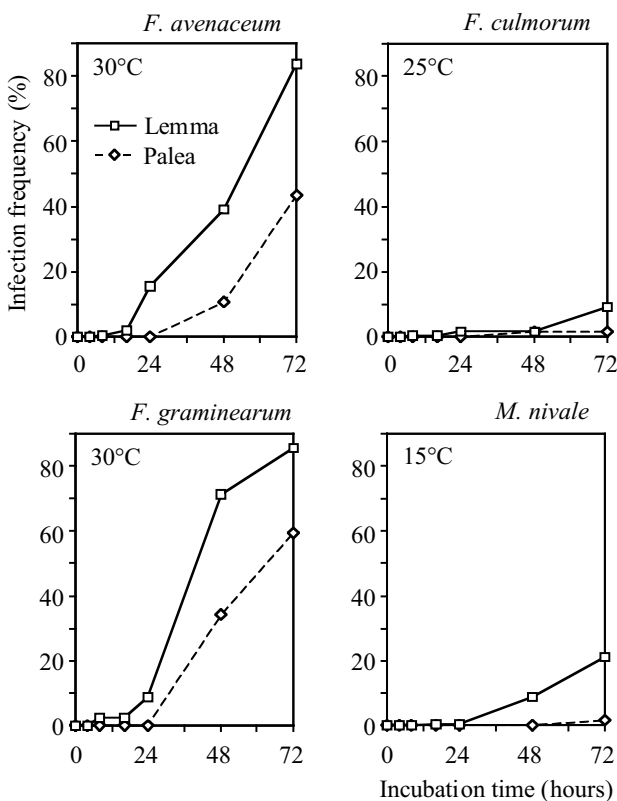


Fig. 2. Frequency of lemma and palea infected by four fungal species causing head blight of wheat, after different times of incubation at the most favourable temperature for infection. The ANOVA performed on the arcsin-square root transformed data showed the significant ($F_{90,672} = 3.22$) effect of the interaction 'fungus x temperature x incubation time x glume type', with $LSD_{0.05} = 4.71$. Data in the graph are true percentages.

The contour plots resulting from the regression equations showed the different response of fungi to changing conditions following inoculation (Fig. 3). *F. graminearum* and *F. avenaceum* showed similar patterns of the contour levels in the range of temperatures between 25.0 and 40.0°C, but the former fungus showed a greater ability to infect wheat tissue below 25.0°C; for both fungi six contour levels were drawn, until the delimitation of the area between 60 and 90% of infected glumes (Fig. 3). After 72 hours incubation, the minimum temperature for infection was 10.0°C for *F. graminearum* and 14.0°C for *F. avenaceum*; the optimum was 29.0 and 28.0°C, respectively, whereas the maximum was very similar (35.0 and 35.5°C, respectively) (Fig. 4). Only three contour levels (0.1 to 3% of infected glumes) were drawn for *M. nivale*; compared to the two previously described fungi, they were shifted over the lowest temperatures, over a narrower range of temperatures (Fig. 3). Minimum and maximum temperatures for infection were 10.0 and 28.5°C, respectively, whereas the optimum was 18.0°C (Fig. 4). The contour plot for *F. culmorum* confirmed the limited ability of the fungus to infect wheat glumes under these environmental conditions: only two contour levels were drawn (0.1 and 1%), over a narrow range of temperatures (Fig. 3). Cardinal temperatures were 16.5°C (minimum), 26.5°C (optimum), and 33.0°C (maximum) (Fig. 4).

Influence of humidity. In the second experiment, spikes were inoculated with the four fungi, incubated for 72 hours, either in the presence of a film of water, in a saturated atmosphere, or without the film of water, under different levels of relative humidity. Infection

Table 3. Frequency (%) of wheat glumes infected by fungi causing head blight under different conditions of incubation.

Fungal species	Temperature (°C)	Relative humidity (%)				
		100 wet ^a	95 dry	85 dry	75 dry	65 dry
<i>F. avenaceum</i>	30	64.81 ^b	0.84	2.14	1.05	6.70
<i>F. culmorum</i>	30	0.94	0.00	1.44	10.21	17.44
<i>F. graminearum</i>	30	73.45	3.13	0.37	0.00	0.55
<i>M. nivale</i>	20	3.13	0.00	0.09	0.55	0.75

^a Conditions of wetting or drying of the spikes.

^a The ANOVA performed on the arcsin-square root transformed data showed the significant ($F_{12,80} = 39.41$) effect of the interaction 'fungus x conditions of incubation', with $LSD_{0.05} = 6.37$. Data in the table are true percentages.

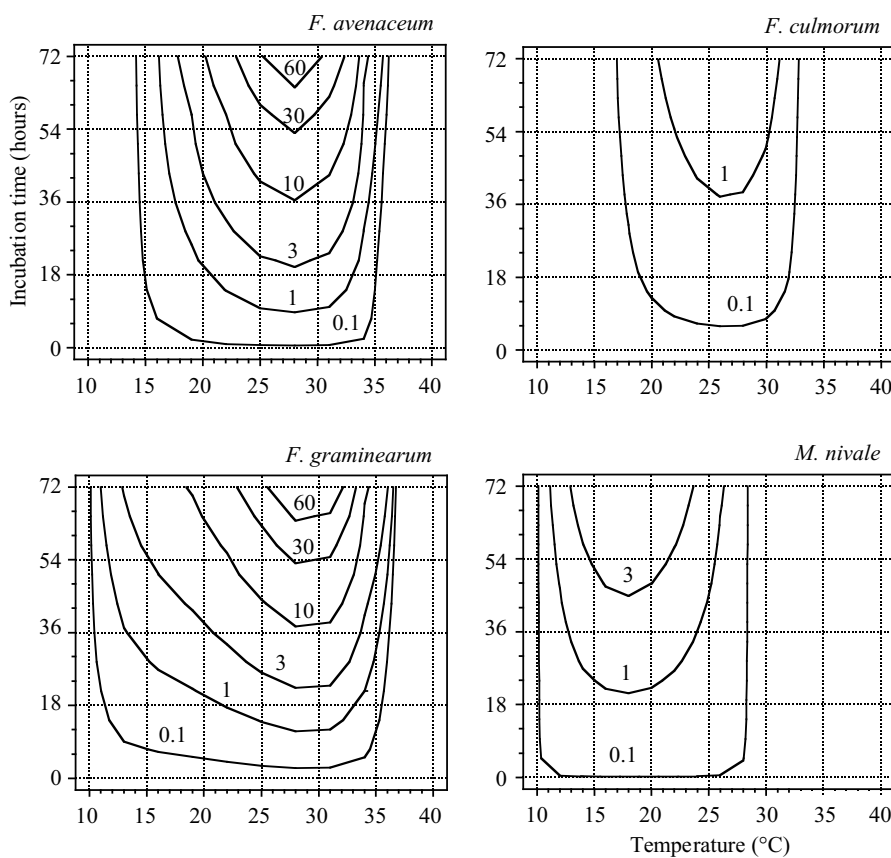


Fig. 3. Contour plot for the regression equations fitting the data on the relationship between temperature, incubation time and the frequency of glumes (palea and lemma) infected by four fungal species causing head blight of wheat. Experimental data are shown in Fig. 1, whereas regression equations are in Table 2. The following contour lines were drawn: 0.1, 1, 3, 10, 30, 60, 90% of infected glumes.

frequency of glumes was significantly ($P < 0.01$) affected by the fungus, by the conditions of humidity, and by the interaction between the two previously mentioned factors; interaction was the most important source of variation, accounting for 41% of total experimental variance. When spikes were incubated wetted at 100% RH, frequency of infected glumes after 72 hours incubation was very high for *F. graminearum* (73.5%) and *F. avenaceum* (64.8%), whereas for *M. nivale* (3.1%) and *F. culmorum* (1.0%) it did not significantly differ from the uninoculated test (0.5%) (Table 3). When spikes were incubated dry, the infection frequency was

significantly influenced by RH. For *F. graminearum* and *M. nivale* the occurrence of infected glumes was occasional at any level of RH. For *F. avenaceum* infection was erratic, dropping to traces between 95.0% and 75.0% RH, and concerning 6.7% of glumes at 65.0% RH. For *F. culmorum*, the infection frequency remained at traces until 85.0% RH, then significantly increased at 75.0% and 65.0% RH (10.2 and 17.4% of infected glumes, respectively). Whereas *F. graminearum* and *F. avenaceum* showed the highest infection ability when spikes were kept wet, *F. culmorum* did this when the spikes were dried and RH reduced.

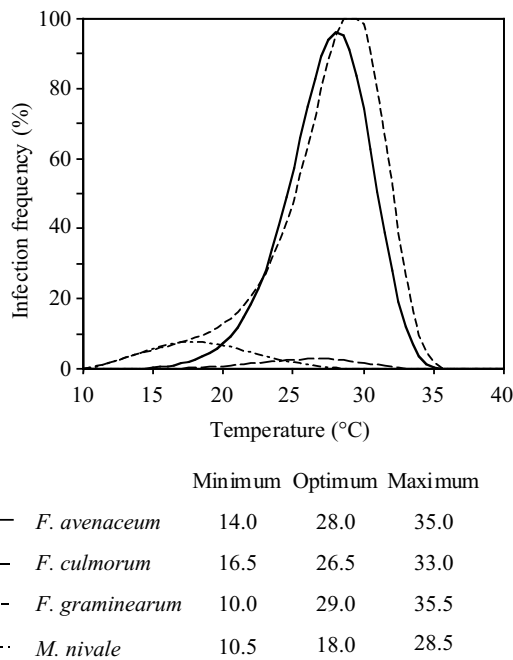


Fig. 4. Frequency of glumes (palea and lemma) infected by four fungal species causing head blight of wheat, after 72 h incubation at different temperatures. Lines were drawn solving the regression equations in Table 2, for incubation $t = 72$. Cardinal temperatures were calculated setting $Y = 0$ (for minimum and maximum temperature) and $Y' = 0$ (for optimum temperature).

DISCUSSION

Knowledge of the environmental conditions favouring infection by the fungal species causing FHB on wheat is crucial for the optimal timing of fungicide application. The results of the present work contribute to defining better the conditions of temperature and humidity that make infection possible, in the case of *F. graminearum*, *F. culmorum*, *F. avenaceum*, and *M. nivale*. In particular, the work (i) produced mathematical equations able to fit accurately the relationships between temperature, time of spike wetting and infection frequency of spike tissues, which were not available in literature, and (ii) quantified the effect of drying of the spikes and of relative humidity, when spikes are dried.

Two aspects of the work evoke discussion. The first point concerns the consistency of the results which were obtained by solving mathematical equations fitting experimental data (Table 3 and Fig. 4) over previous knowledge. The second point relates to the possible use of results from the present work in disease management.

For *F. graminearum*, information about environmen-

tal conditions favouring infection was already available in literature. Although exact comparison between these previous findings and the results of the present work is not possible, because the experimental conditions were different, there was a substantial consistency between them. Andersen (1948) found that the optimum temperature for infection and disease development was 25.0°C. Little or no infection occurred at 15.0°C. Disease incidence increased as temperature increased from 20.0 to 30.0°C. The moist period required for infection depended on temperature: it was 24 h at 25.0°C, and ranged from 36 to 72 h at 20.0°C. Pugh *et al.* (1933) found that at 25.0°C, 18% and 77% of spikes become infected after 36 and 48 h of continuous wetness, respectively. At 20.0°C, 60 h of wetness were required to infect 30% of heads, whereas at 15.0°C negligible infection occurred. Atanasoff (1920) observed that, in inoculated spikes, disease symptoms appeared within 6 day when conditions were wet, whereas when conditions were dry, symptoms did not appear until the first rain or heavy dew. Andersen (1948) found that interruption of the wet period after inoculation significantly reduced the incidence of infected heads. These previous findings are in general agreement with the contour plot of Fig. 4 and the data in Table 3.

For *F. avenaceum*, *F. culmorum*, and *M. nivale*, no specific works have been published on the effect of temperature and humidity on wheat spike infection. Nonetheless, there was a general agreement between the knowledge of the ecological requirements of these species and the present results. A few differences between the optimal temperatures for growth found by Domsch *et al.* (1980) and the ones for wheat glume infection resulting from the present work were observed.

A system forecasting the time of infection establishment and incidence of the disease could be a useful tool in improving the efficacy of fungicide application. Parry *et al.* (1995) emphasised that efficacy of aerial application of fungicides is often of doubtful effect, due to three main reasons: the inherent activity of molecules used, the possible resistance to fungicides in pathogen populations, and problems associated with the timing of fungicide application. Some reports published in the '90s demonstrated that successful control of the disease can be obtained using prochloraz, flusilazole, tebuconazole or strobilurin containing fungicides (Hutcheon and Jordan, 1992; Wainwright *et al.*, 1992; Matthies *et al.*, 2000). Tebuconazole was able to reduce disease severity by up to 70% (Obst *et al.*, 1992; Suty and Mauler-Machnik, 1997) and contamination with mycotoxins (Homdork *et al.*, 2000; Matthies and Buchenauer, 2000); it showed a broad spectrum of action (Hutcheon and Jordan, 1992) and was active also in early curative

treatments (Santori *et al.*, 1999; Matthies and Buchenauer, 2000). Thus, notwithstanding that effective molecules are now available, the difficulty in determining the optimum time of application still persists. The results from the present work can contribute to overcoming this impasse. In fact, they allow to define the establishment of infection for each of the fungal species considered in the present work, based on the conditions of temperature and humidity occurring after inoculation.

ACKNOWLEDGEMENTS

This research was supported, in part, by funding from the CNR (Consiglio Nazionale delle Ricerche, coordinate project EPIFUS) and from the Fondazione Invernizzi. The Authors wish to thank Prof. S. Frisullo for confirming identification of the four *Fusarium* species.

REFERENCES

- Andersen A.L., 1948. The development of *Gibberella zeae* headblight of wheat. *Phytopathology* **38**: 595-611.
- Atanasoff D., 1920. *Fusarium* blight (scab) of wheat and other cereals. *Journal of Agricultural Research* **20**: 1-32.
- Bai G.H., Shaner G., 1991. Effect of moist period on response of wheat cultivars to infection by *Fusarium graminearum*. *Phytopathology* **81**: 1145-1146.
- Bechtel D.B., Kaleikan L.A., Gaines R.L., Seitz L.M., 1985. The effects of *Fusarium graminearum* infection on wheat kernels. *Cereal Chemistry* **20**: 272-290.
- Bottalico A., Logrieco A., Visconti A., 1989. *Fusarium* species and their mycotoxins in infected cereals in the field and in stored grains. In: Chelkowski J. (ed.). *Fusarium-mycotoxins, taxonomy and pathogenicity*, pp. 85-120. Elsevier, Amsterdam, The Netherlands.
- Burgess L.W., Liddel C.M., Summerell B.A., 1988. Laboratory manual for *Fusarium* research. II Ed. University of Sydney, Sydney, Australia.
- Corazza L., Balmas V., Santori A., Magnotta A., 1997. I principali agenti della fusariosi della spiga in Italia. *L'Informatore Agrario* **53**: 55-57.
- Dickson J.G., Johann H., Wineland G., 1921. Second progress report on the *Fusarium* blight (scab) of wheat. *Phytopathology* **11**: 35.
- Domsch K.H., Gams W., Anderson T.H., 1980. Compendium of soil fungi. London Academic Press, London, UK.
- Hoagland D.R., Arnon D.J., 1950. The water-culture method for growing plants without soil. California Agricultural Experimentation Station, circular no. 347.
- Homdork S., Fehrmann H., Beck R., 2000. Effects of field application of tebuconazole on yield, yield components and the mycotoxin content of *Fusarium*-infected wheat grain. *Journal of Phytopathology* **148**: 1-6.
- Hutcheon J.A., Jordan V.W.L., 1992. Fungicide timing and performance for *Fusarium* control in wheat. In: *Brighton Crop Protection Conference, Farnham 1992*, 633-638.
- Matthies A., Buchenauer H., 2000. Effect of tebuconazole (Folicur R) and prochloraz (Sportak R) treatments on *Fusarium* head scab development, yield and deoxynivalenol (DON) content in grains of wheat following artificial inoculation with *Fusarium culmorum*. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **107**: 33-52.
- Matthies A., Menck B.H., Bleiholder H., 2000. A comparative study into the effects of strobilurin containing and azole fungicides on the content of deoxynivalenol (DON) in wheat samples of 1999. Initial results. *Gesunde-Pflanzen* **52**: 26-32.
- McMullen M., Jones R., Gallenberg D., 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. *Plant Disease* **81**: 1340-1348.
- Nelson P.E., Toussoun T.A., Marasas W.F.O., 1983. *Fusarium* species. An illustrated manual for identification. Pennsylvania State University Press, University Park Pennsylvania, USA.
- Obst A., Lepschy J., Huber G., 1992. Zur gezielten Bekämpfung der Ährenfusariosen bei Weizen – Beobachtungen und Versuchsergebnisse aus Bayern. *Gesunde Pflanzen* **44**: 40-47.
- Orsi C., Chiusa G., Rossi V., 1994. Ulteriori indagini sulla micoflora delle cariossidi di frumento duro, in rapporto alla volpatura. *Petria* **4**: 225-236.
- Pancaldi D., Torricelli R., 1998. Presenza di specie di *Fusarium* su cariossidi di frumento tenero in Emilia-Romagna nel triennio 1994-'96. *Informatore Fitopatologico* **54**: 46-50.
- Parry D.W., Jenkinson P., McLeod L., 1995. *Fusarium* ear blight (scab) in small grain cereals - a review. *Plant Pathology* **44**: 207-238.
- Porta-Puglia A., Santorelli S., 1997. Stato sanitario delle sementi di frumento prodotte nel 1996. *L'Informatore Agrario* **53**: 73-74.
- Pugh G.W., Johann H., Dickson J.G., 1933. Factors affecting infection of wheat heads by *Gibberella saubinetii*. *Journal of Agricultural Research* **46**: 771-797.
- Rossi V., Frisullo S., Manici L.M., 1991. Indagine sulla micoflora delle spighe di frumento duro, in rapporto alla volpatura delle cariossidi. *Petria* **1**: 37-50.
- Santori A., Balmas V., Corazza L., 1999. Fusariosi, la risorsa tebuconazolo. *Terra e Vita* **40**: 57-61.
- Suty A., Mauler-Machnik A., 1997. *Fusarium* ear blight on wheat – Epidemiology and control of *Gibberella zeae*, the

- teleomorph of *Fusarium graminearum*, with Folicur. In: Dehne H.W., Adam G., Diekmann M., Frahm J., Mauler-Machnik A., Van Halteren P. (eds.). *Diagnosis and Identification of Plant Pathogens*, Bonn 1996, 243-246.
- Tuite J., Shaner G., Everson R.J., 1990. Wheat scab in soft red winter wheat in Indiana in 1986 and its relations to some quality measurements. *Plant Disease* **74**: 959-962.
- Wainwright A., Jeitner J., Cazin-Bourguigno P., 1992. Reduction in the wheat ear disease complex with tebuconazole sprays. In: *Brighton Crop Protection Conference*, Farnham 1992, 621-626.
- Wiese M.V., 1977. Compendium of wheat diseases. The American Phytopathological Society, St. Paul, Minnesota, USA.
- Zadoks J.C., Chang T.T., Konzak B.F., 1974. A decimal code for the growth stages of cereals. *Weed Research* **14**: 415-421.

Received 22 November 2000

Accepted 13 June 2001