European Journal of Plant Pathology **104:** 37–48, 1998.

Influence of crop management on eyespot development and infection cycles of winter wheat

Nathalie Colbach and Laurent Saur
URI-GC, INRA, BP 29, 35650 Le Rheu, France; Address for correspondence: Station d'Agronomie, INRA, 17 rue Sully, BV1540, 21034 Dijon Cedex, France

Accepted 29 September 1997

Key words: cultural practices, disease progress equation, infection cycles, *Pseudocercosporella herpotrichoides*, *Triticum aestivum*

Abstract

Wheat was assessed at four crop growth stages for eyespot (anamorph *Pseudocercosporella herpotrichoides*, teleomorph *Tapesia yallundae*) in a series of field trials that studied the effects on disease frequency of five wheat management techniques (sowing date and density, nitrogen fertiliser dose and form, removal/burial of cereal straw). An equation expressing disease level as a function of degree-days was fitted to the observed disease levels. This equation was based on eyespot epidemiology and depended on two parameters illustrating the importance of the primary and the secondary infection cycles respectively. Cultural practices were classified according to the importance of their effects on disease, and these effects could be related to infection cycles and host plant architecture. Sowing date had the earliest and strongest effect; early sowing always increased disease frequency through the primary infection cycle, and its influence on the secondary cycle was variable. Disease frequency was increased by high plant density and/or a low shoot number per plant through primary infection; the secondary cycle was, however, decreased by a low shoot number per plant, which reduced late disease development at high plant density. High nitrogen doses increased disease levels and the severity of both infection cycles, but this effect was partly hidden by a simultaneous stimulation of tillering and thus an indirect decrease of disease incidence. When significant, ammonium (vs ammonium nitrate) fertiliser decreased eyespot levels and infection cycles whereas straw treatment (burial vs removal of straw from the previous cereal crop) had no effect.

Introduction

The eyespot fungus (anamorph *Pseudocercosporella herpotrichoides* (Fron) Deighton; teleomorph *Tapesia yallundae* Wallwork & Spooner) infects stem bases of most winter cereals, interfering with water and nutrient transport and sometimes causing plants to lodge. The disease can reduce yield substantially (Scott and Hollins, 1974; Clarkson, 1981; Schaub and Schlösser, 1982; Meunier, 1984). As the fungus regularly develops resistance to commercial fungicides (Maraite and Weyns, 1986; Leroux and Gredt, 1988; Cavelier et al., 1992), chemical control must be augmented with other management strategies. Furthermore, the growing importance of environmental concerns will necessitate a limitation in pesticide use. Therefore, the effect of cropping systems on eyespot must be studied in order to optimise crop management to limit disease development and yield losses.

Crop rotation, which has a major influence on *P. herpotrichoides*, has been studied previously both by ourselves (Colbach et al., 1994; Colbach and Huet, 1995; Colbach and Meynard, 1995) and numerous other authors (e.g. Glynne and Slope, 1959; Maenhout, 1975; Steinbrenner and Höflich, 1984; Schulz et al., 1990; Polley and Thomas, 1991). In this paper, we will analyse exclusively the effects of crop management on eyespot.

The effect of weather conditions on disease development has already been extensively studied (Schrödter and Fehrmann, 1971 a, b; Rapilly et al., 1979), but the interaction with crop management is

Table 1. Cultural practices evaluated for management of eyespot of wheat, conducted in France in 1991–1993

Treatment	Level	Experiment site			
		Le Rheu 92	Le Rheu 93	La Verrière 92	Chartres 92
Sowing date	early	17 Oct. 91	16 Oct. 92	11 Oct. 91	8 Oct. 91
	late	26 Nov. 91	24 Nov. 92	6 Nov. 91	7 Nov. 91
Plant density[a]	high	231	194/108[b]	236	228
(plants/m^2)	low	140	138/74[b]	70	179
Total available	high	325	325	380	290
nitrogen (kg/ha)[c]	low	235	230	300	200
Nitrogen form	NH_4^+	ammonium sulphate	ammonium sulphate	none	urea+ammonium sulphate
	NH_4NO_3	ammonium nitrate	ammonium nitrate	ammonium nitrate	ammonium nitrate
Straw treatment		buried	buried	buried	buried
		baled	baled	burnt	baled

[a] Seed depth was 2-3 cm and row spacing was 16 cm.
[b] The first density was observed on early, the second on late sown plots.
[c] Total nitrogen = available soil nitrogen + mineralisation + nitrogen applications. The amount of nitrogen fertiliser applied was calculated according to the predictive balance sheet method (Rémy and Hébert, 1977). Nitrogen fertiliser was applied as top dressing twice in the season at GS 15 and 30.

Table 2. Description of the experimental sites used to evaluate cultural practices for management of eyespot of wheat in experiments conducted in France during 1991–1993

Experiment site	Longitude	Latitude	Altitude	Soil texture			Mean annual temperature (°C)			Mean annual precipitation (mm)		
				loam (%)	silt (%)	sand (%)	Mean[a]	91–92[b]	92–93[b]	Mean[a]	91–92[b]	92–93[b]
Chartres	1°31′	48°28′	156 m	22–24	66–68	5	10.3	10.8		581	340	
Le Rheu	1°43′	48°01′	34 m	15	70	15	10.2	11.6	11.5	714	616	675
La Verrière	2°01′	48°46′	168 m	14–16	29–32	38–42	10.1	10.8		639	497	

[a] Mean over 30 years.
[b] Annual means for 1991–92 and 1992–93 were calculated from August 1991 to July 1992 and from August 1992 to July 1993, respectively.

poorly understood. Several authors have studied this interaction to determine if and when to use fungicides against eyespot (e.g. Frahm and Knapp, 1986; Sieberasse and Fehrmann, 1987; Groll and Gutsche, 1989), but their work was based on surveys and not on closely monitored field trials. They could thus not study disease development or relate the effects of cultural practices to host or pathogen characteristics.

In this study, disease development was monitored in field trials in which various crop management techniques were combined in order to (a) evaluate crop management influence on eyespot build-up, (b) classify cultivation techniques according to their impact on disease, and (c) study the interaction between these factors.

Material and methods

Experimental plan

Winter wheat was visually assessed for eyespot in a multifactorial field trial design combining five cultural practices (Table 1). The experiment was conducted in three different locations (Le Rheu, La Verrière, Chartres) in 1991-92, and again in Le Rheu in 1992-93; locations were chosen to provide a combination of different soil and climatic conditions (Table 2). To maximise disease risk, the crops preceding the assessed wheat at each site were either winter wheat or winter barley, the latter also being a host for eyespot.

For each cultural practice, two levels were chosen (Table 1): sowing date (early vs late), sowing density (high vs low), total nitrogen dose (high vs low), nitrogen fertiliser form (low vs high ammonium con-

tent) and removal/burial of straw originating from the previous crop. Most treatments were arranged factorially except for the combinations of low nitrogen dose × buried straw and of high ammonium nitrogen × removed straw. At La Verrière, no high ammonium nitrogen was used, but the low nitrogen × buried straw combination was included. Thus, there were always 16 different combinations of experimental factors. At three locations (Le Rheu, 1992, Le Rheu, 1993, Chartres), a balanced 4-block-design was used and every experiment had of 64 plots; at La Verrière, the design was composed only of three blocks and 48 plots. Each plot was 10 m × 6 m.

Cultural practices applied uniformly to all plots were as follows. The eyespot susceptible cultivar Soissons was used in each experiment. Fields were ploughed and harrowed the day before or on the day of sowing and a herbicide (Trifluralin+Linuron at sowing, Bifenox+Ioxynil+Mecoprop-P in spring) was sprayed the day after sowing and again in the spring. No fungicides were applied for stem-base diseases but foliar diseases, septoria diseases and rusts which usually occurred in the areas of experimentation, were controlled with fungicide applications at heading (Tebuconazole).

Sampling and measurements

Wheat was visually assessed for eyespot at four growth stages: 5-leaf stage (GS 15), pseudostem erect (GS 30), first spikelets visible (GS 50) and early dough (GS 80) (Zadoks et al., 1974). Plants were sampled together with roots and soil from four randomly chosen sub-plots, each measuring 25 cm × 2 rows, and separated after washing off the soil; eight plants were sampled from each subplot and the percentage of diseased plants was calculated. At GS 80, the *P. herpotrichoides* varieties isolated from stems with symptoms were identified *in vitro*. For each plot, 30 diseased shoots were surface-sterilised (1 min in 1% sodium hypochlorite solution), then transferred to potato-dextrose-agar. After 10 days incubation at 20 °C, form, size and colour of the colonies were used to distinguish *P. herpotrichoides* var. *herpotrichoides* from *P. herpotrichoides* var. *acuformis* (Nirenberg, 1981).

The number of plants m^{-2} was determined and at each assessment stage together with the number of shoots per plant (tillers + the main stem). At GS 15 and 30, all tillers were counted; at the last two stages, only tillers with ears were considered. At GS 30, plants were also analysed for nitrogen content, which was compared to optimum nitrogen content using the reference curve established by Justes et al. (1994).

Statistics

Linear model

To analyse eyespot levels at each assessment time and location, we used analyses of covariance. All experimental factors were treated as qualitative variables, except plant density which was introduced as a quantitative variable.

model #1: %of plants with eyespot = constant + sowing date effect + nitrogen rate effect + fertiliser form effect + straw treatment effect + block effect + assessor effect + a × plants m^{-2} + b × shoots $plant^{-1}$ + interaction sowing date∗nitrogen rate + interaction sowing date∗fertiliser form + interaction sowing date∗straw treatment + interaction sowing date∗plants m^{-2} + interaction sowing date∗shoots $plant^{-1}$ + error

Only the interactions of the major factor, i.e. sowing date, with the other cultural practices were analysed to keep the models as simple as possible. Nitrogen rate and form effects were used only in the models corresponding to those stages that were subsequent to the first nitrogen fertiliser application, i.e. GS 30 (GS 50 for Chartres where nitrogen rate and form did not differ at the first application). An assessor effect was introduced into the models to take into account the subjectivity due to different persons assessing disease levels. In order to study the relationship between plant architecture and eyespot, the number of shoots per plant T_i was introduced as a supplementary covariable. The effect of crop management on the number of shoots per plant was studied in a second series of models:

model #2: shoots $plant^{-1}$ = constant + sowing date effect + nitrogen rate effect + fertiliser form effect + straw treatment effect + block effect + assessor effect + a × plants m^{-2} + interaction sowing date∗nitrogen rate + interaction sowing date∗fertiliser form + interaction sowing date∗straw treatment + interaction sowing date∗plants m^{-2} + error

The final models contained only those factors and/or covariables for which the probability values of the statistical zero hypothesis test were less than $\alpha = 0.05$. If these values were higher than $\alpha = 0.05$, the contribution due to the factor or covariable was considered to be not significant. The sum of squares used to calculate

the probability values for each factor or covariable was adjusted to all terms present in the model and thus did not depend on their order of appearance in the model. All analyses were done with the General Linear Model (GLM procedure) of the SAS software (Statistical Analysis System, SAS Institute Inc, 1989).

Disease build-up as a function of thermal time

Eyespot development as a function of time was studied with the help of the kinetic equation established by Colbach and Meynard (1995) and based on eyespot epidemiology. The basic assumptions were as follows: (a) there are two possible sources of inoculum: primary inoculum produced by host residues close to the soil surface and secondary inoculum produced on diseased living plants; (b) each inoculum type has its own infection rate. The relation between the percentage of diseased plants y and time t expressed as a sum of degree-days from sowing is:

$$model\#3: \; y = \frac{1 - e^{-(c_1+c_2)\cdot t}}{1 + \frac{c_2}{c_1} \cdot e^{-(c_1+c_2)\cdot t}}$$

in which c_1 is the infection rate for primary inoculum and c_2 is the infection rate for secondary inoculum. Equation #3 was adjusted to eyespot build-up (corrected for assessor and block influences if these were significant in model #1) for every experimental treatment on each site. We used the Non-Linear Model (NLIN) procedure of SAS based on minimisation of square sums weighted by the inverse of the variance corresponding to each assessment stage. After a series of iterations, this adjustment estimated the parameters c_1 and c_2 for each experimental treatment.

Classification of the cropping systems according to the primary and secondary infection parameters

On each of the two groups of parameter values, we tested a linear model (analysis of covariance) to explain the parameter value for each site, as a function of the various factors and covariables used in model #1. The initial model was as follows:

model #4: Estimate of c_i = constant + sowing date effect + nitrogen rate effect + fertiliser form effect + straw treatment effect + a $\times$ plants m^{-2} + b $\times$ shoots $plant^{-1}$ + interaction sowing date$*$nitrogen rate + interaction sowing date$*$fertiliser form + interaction sowing date$*$straw treatment + interaction sowing date$*$plants m^{-2} + interaction sowing date$*$shoots $plant^{-1}$ + error

This model contains neither assessor nor block effects, as we only have one set of parameters for each experimental treatment. As before, the final model only contained the statistically significant factors and/or covariables. If the variances were not homogeneous, the Box and Cox transformation (Box et al., 1978) was used to homogenise variance; if the linear regression *ln(variance(parameter)) = a + b • ln(parameter)* was significant, the parameter c_i was transformed as follows:

$$c_{transformed} = c^{1-b/2}$$

Results

The number of shoots per plant

Plant density and sowing date were the most frequently significant factors affecting tillering (Table 3). Early sowing at high density increased the number of shoots per plant. The other cultural practices had less effect. High vs low nitrogen and ammonium vs ammonium nitrate increased tillering whereas the impact of straw treatment was variable.

Eyespot incidence

The percentage of diseased plants was highest at La Verrière and lowest at Chartres (Table 4). These were also the two locations where the *P. herpotrichoides* population was nearly entirely composed of *P. herpotrichoides* var. *herpotrichoides*. The proportion of *P. herpotrichoides* var. *acuformis* was high in both years at Le Rheu, but in 1993, the *in vitro* identification also revealed a high proportion of shoots with both eyespot varieties. Sowing date was the most frequently significant factor for determining eyespot incidence, and early sowing always increased disease frequency (Table 5). When sowing date had no effect on disease, no other factor was significant. The only exception is the last stage at Le Rheu 1993; at this location, there was a considerable difference in densities between early and late sowing (Table 1) and it was thus difficult to separate the effect of these two factors on eyespot. Plant density was rarely positively correlated to eyespot frequency (Table 5). However, disease was frequently influenced by the covariable shoots per plant, which was itself intimately related to plant density (Table 3). Correlation between tillering and disease was always

Table 3. Regression estimates for the effect of wheat cultural practices on the number of shoots per plant[a]

Growth stage	Cultural practice	Level	Experiment site			
			Le Rheu 92	Le Rheu 93	La Verrière 92	Chartres 92
15[b]	Sowing date	early	0.3		− 0.4	0.2
		late	− 0.3	[c]	0.4	− 0.2
	Plants m^{-2}	quantitative variable			− 0.002[c]	
					− 0.006[c]	
	r^2		0.34	0	0.72	0.40
30	Sowing date	early	3.1		2.6	2
		late	− 3.1		− 2.6	− 2
	Plants m^{-2}	quantitative variable	− 0.025[d]	− 0.0009	− 0.009[d]	− 0.013[d]
			− 0.002[d]		− 0.006[d]	*ns*[d,e]
	Fertiliser form	NH_4	− 0.2		*nd*[f]	
		NO_3NH_4	0.2			
	Straw treatment	burial	− 0.9[g]	0.14	*na*[h]	*na*[h]
		removal	0.9	− 0.14		
	r^2		0.91	0.59	0.84	0.75
50	Sowing date	early	0.7		1.3	0.5
		late	− 0.7		− 1.3	− 0.5
	Plants m^{-2}	quantitative variable	− 0.008[c]	− 0.008	− 0.04[c]	− 0.007[c]
			− 0.004[c]		− 0.03[c]	− 0.004[c]
	Total nitrogen	high	0.1	0.3		
		low	− 0.1	− 0.3		
	Straw treatment	burial			− 0.5	− 0.1[i]
						0.3[i]
		removal			0.5	0.1[i]
						− 0.3[i]
	r^2		0.82	0,60	0.90	0.83
80	Sowing date	early	0.2		3.4	0.6
		late	− 0.2		− 3.4	− 0.6
	Plants m^{-2}	quantitative variable	− 0.009	− 0.006	− 0.5[d]	− 0.007[d]
					− 0.2[d]	− 0.003[d]
	Total nitrogen	high		0.13		
		low		− 0.13		
	Straw treatment	burial			− 0.6	
		removal			0.6	
	r^2		0.69	0.54	0.94	0.85

[a] Estimates are for model #1 shown in text; effects of assessor and block on disease are not shown.
[b] Decimal growth stage of wheat on Zadoks scale (Zadoks et al., 1974).
[c] Late sown plots were not assessed at this stage.
[d] The interaction between sowing date and plant density was significant; the first number is the estimate of the correlation parameter of shoots per plant and plant density on early sown plots, the second number the correlation parameter for late sown plots.
[e] This number was not significant from zero at P = 0.05.
[f] There were no plots with NH_4 on this site.
[g] The interaction between sowing date and straw treatment was significant; the numbers are the estimated effects of straw treatment on early sown plots.
[h] The plots with buried straw were not assessed at these locations.
[i] The interaction between sowing date and straw treatment was significant; the first number is the estimated effects of straw treatment on early sown plots, the second on late sown plots.

Table 4. Incidence of eyespot and varieties of *Pseudocercosporella herpotrichoides* at each site

Stage	Percentage of plants with eyespot (mean ± standard error)[a]			
	Le Rheu 92	Le Rheu 93	La Verrière 92	Chartres 92
15	2 ± 3	11 ± 9	7 ± 4	0.7 ± 1.1
30	9 ± 11	14 ± 35	37 ± 35	0.4 ± 1.4
50	44 ± 30	28 ± 20	89 ± 11	29 ± 21
80	85 ± 13	70 ± 19	99 ± 1	42 ± 25
	Percentages of eyespot lesions from which *P. herpotrichoides* varieties were isolated[b]			
var. *herpotrichoides*	40 ± 15%	67 ± 27	100 ± 0%	100 ± 0.03%
var. *acuformis*	63 ± 17%	57 ± 27	0.01 ± 0.03%	0.02 ± 0.03%

[a] Means and standard errors for all plots at a given site.
[b] Sums higher than 100% are due to symptoms on which both varieties were identified.

Table 5. Regression estimates for the effect of wheat cultural practices on the percentage of plants with eyespot[a]

Growth stage	Cultural practice	Level	Experiment site			
			Le Rheu 92	Le Rheu 93	La Verrière 92	Chartres 92
15[b]	Sowing date	early	2		1.6	
		late	− 2	[c]	− 1.6	
	Plant density	quantitative variable			0.03	
	r^2		0.37	0	0.58	0
30	Sowing date	early	6.9		24.7	
		late	− 6.9		− 24.7	
	Shoots plant^{-1}	covariable			− 13.8	
	r^2		0.39	0	0.65	0
50	Sowing date	early	20.7	3.9		20.9
		late	− 20.7	− 3.9		− 20.9
	Shoots plant^{-1}	covariable	− 10.2	− 13.6	− 1	− 8.6
	Fertiliser form	NH_4	− 9.4[d]		*nd* [e]	
		NO_3NH_4	9.4[d]			
	Total nitrogen	high			2.6	4.6[f]
		low			− 2.6	− 4.6[f]
	r^2		0.90	0.56	0.53	0.70
80	Sowing date	early	8.9			22.9
		late	− 8.9			− 22.9
	Plant density	quantitative variable		0.14		
	Shoots plant^{-1}	covariable	2.5			− 11.4
	Fertiliser form	NH_4		− 3.9	*nd*	
		NO_3NH_4		3.9		
	r^2		0.65	0.59	0	0.78

[a] Estimates are for model #2 shown in text; effects of assessor and block on disease are not shown.
[b] Decimal growth stage of wheat on Zadoks scale (Zadoks et al., 1974).
[c] Late sown plots were not assessed at this stage.
[d] The interaction between sowing date and fertiliser form was significant; the numbers are the estimated effects of fertiliser form on early sown plots.
[e] There were no plots with NH_4 on this site.
[f] The interaction between sowing date and nitrogen rate was significant; the numbers are the estimated effects of nitrogen rate on late sown plots.

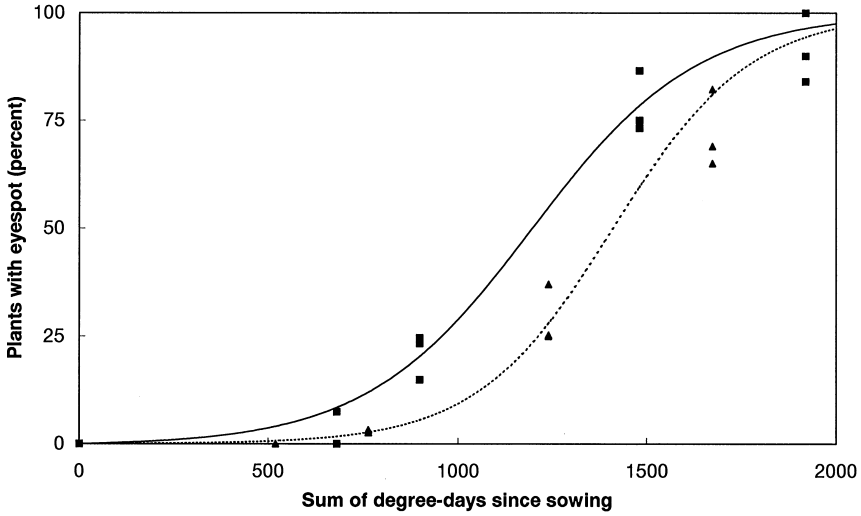

Figure 1. Eyespot build-up as a function of time. Example of fitting model #3 to measured data at Le Rheu 92 for treatments with early- (— = simulated and ■ = measured data) and late-starting disease evolutions (- - - = simulated and ▲ = measured data).

negative except at GS 80 at Le Rheu 1992. The effects of the nitrogen amount and of the fertiliser form were significant on only two occasions (Table 5). In these cases, disease was more frequent on plots fertilised with ammonium nitrate than with ammonium sulphate and eyespot was increased by a high compared with low nitrogen amount. The impact of these two factors on plant nitrogen content was also analysed: fertiliser form did not influence nitrogen content and the increase in nitrogen content on plots with high nitrogen rates was not associated with an effect on disease. The burial vs. removal of cereal straw had no effect on eyespot.

Disease evolution as a function of 'cumulative degree-days'

The kinetic equations usually fitted well to the observed disease data (Figure 1); at the two sites with high eyespot level: mean r^2 was 0.99 (standard-error = 0) for La Verrière, 0.98 for Le Rheu 92 (standard error = 0.01) and 0.91 for Le Rheu 93 (standard error = 0.07). The r^2 value was considerably less and more variable at Chartres where eyespot level was low: r^2 = 0.68 with a standard error of 0.20. Residuals were normally distributed, except once at Le Rheu 92 and at Le Rheu 93 and twice at Chartres.

The estimates of parameter c_1 were greatest at La Verrière and least at Chartres (Table 6), as was eyespot frequency (Table 4). The site ranking was different for parameter c_2: values were greater at La Verrière and Chartres, which were the sites with nearly no *P. herpotrichoides* var. *acuformis*, than at Le Rheu where this variety was frequent. Early sowing always increased the values of c_1 (Table 7) whereas the effect of sowing date on parameter c_2 was variable (Table 8). Effects of plant density and shoot number were less frequently observed than effects of sowing date. On these occasions, an increase in plant density resulted in an increase of both c_1 and c_2. The shoot number per plant only influenced c_2; the covariable and the parameter values then were positively correlated. The effects of nitrogen amount and fertiliser form were seldom significant. Increasing nitrogen amounts were however found to increase both c_1 and c_2 whereas fertiliser form influenced only the second parameter which was then higher on plots with ammonium nitrate vs ammonium sulphate. Straw treatment was again without effects.

Discussion

These trials produced several results that are consistent with previous reports, mainly concerning site ranking (Rapilly et al., 1979), disease increase in case of early sowing (Hollins and Scott, 1980; Steinbrenner and Seidel, 1982; Schulz et al., 1990; Groll and Luzi, 1991; Polley and Thomas, 1991) and high plant density (Glynne, 1951; Salt, 1955; Huet, 1986; Groll and Luzi, 1991), and the absence of any effect of straw treat-

Table 6. Mean and standard error values for the parameters c_1, associated to the primary infection cycle, and c_2, associated to the secondary infection cycle of eyespot

Parameter	Experiment site			
	Le Rheu 92	Le Rheu 93	La Verrière 92	Chartres 92
c_1	$1.6 \times 10^{-5} \pm 1.3 \times 10^{-5}$	$1.2 \times 10^{-5} \pm 1.7 \times 10^{-5}$	$1.9 \times 10^{-5} \pm 1.2 \times 10^{-5}$	$1.2 \times 10^{-6} \pm 1.9 \times 10^{-6}$
c_2	$4.3 \times 10^{-3} \pm 8.7 \times 10^{-4}$	$4.4\,10^{-3} \pm 1.9 \times 10^{-3}$	$5.9 \times 10^{-3} \pm 1.4 \times 10^{-3}$	$5.6 \times 10^{-3} \pm 1.3 \times 10^{-3}$

Table 7. Regression estimates for the effect of wheat cultural practices on disease progress model parameter c_1, which is associated to primary eyespot development on wheat[a]

Cultural practice	Level	Experiment site[b]			
		Le Rheu 92	Le Rheu 93	La Verrière 92	Chartres 92
Sowing date	early	0.057	0.0012	0.0015	0.01
	late	− 0.057	− 0.0012	− 0.0015	− 0.01
Plant density	quantitative variable	0.00047[c]	0.0003[c,d]	0.000029[c]	0.00069[d]
Total nitrogen	high	0.012[e]			
	low	− 0.012			
r^2		0.72	0,42	0.76	0.47

[a] Estimates are for model #4 shown in text.
[b] For each site, a Box and Cox transformation was necessary. The transformations were $c_1^t = c_1^{(1-1.61/2)}$ at Le Rheu 92, $c_1^t = c_1^{(1-1.53/2)}$ at Le Rheu 93, $c_1^t = c_1^{(1-1.06/2)}$ at La Verrière and $c_1^t = c_1^{(1-1.51/2)}$ at Chartres.
[c] The interaction between sowing date and plant density was significant. At Le Rheu 92 and La Verrière 92, the number is the estimate of the correlation parameter on late sown plots; at Le Rheu 93 on early sown plots.
[d] This effect is only significant at P = 0.14 and 0.09 at Le Rheu 93 and Chartres 92 respectively, but without its presence no significant model would have been found.
[e] The interaction between sowing date and nitrogen rate was significant the numbers are the estimated effects of nitrogen rate on early sown plots.

ment effects (Shipton, 1972; Steinbrenner and Seidel, 1982; Herman, 1985, 1986). However, even for these rather well investigated factors, we were able to draw some new conclusions. For instance, our site ranking obtained for final eyespot incidence, with highest levels at La Verrière and lowest at Chartres, is not entirely consistent with that given by Rapilly et al. (1979); according to these authors, disease frequency should have been higher at Le Rheu than at La Verrière as the climate is milder and more humid at the first location compared to the second. This difference could not be explained by the initial soil infectivity. At Le Rheu, both the previous crop and the one before this were hosts whereas at La Verrière and also at Chartres, only the immediately preceding crop was a host. However, Rapilly et al. (1979) worked only with *P. herpotrichoides* var. *Herpotrichoides*, whereas the pathogen populations found on our trials at Le Rheu consisted mainly of *P. herpotrichoides* var. *acuformis*. This variety is reported to be less aggressive (Higgins and Fitt, 1985; Creighton et al., 1989) and to grow more slowly *in planta* (Cavelier et al., 1987; Mauler and Fehrmann, 1987 a, b; Moreau et al., 1990), and its frequency on that site might explain the lower final eyespot incidence and also the lesser importance of the primary infections at this location. Furthermore, site ranking for the secondary cycle was also inconsistent with the report by Rapilly et al. This apparent disagreement could also be at least partly related to the difference in pathogen varieties. But the second reason is of a different nature: Rapilly et al. (1979) based their classification on the frequency of pre-winter sporulation-infection conditions whereas the secondary cycle takes place mostly during spring (Moreau et al., 1990) and is therefore seldom influenced by pre-winter climatic conditions.

The effect of sowing date on eyespot does not seem to be as straightforward as indicated in the literature. The frequently given reason for disease increase related to early sowing, i.e. an increase in the favourable pre-winter infection period, would also explain the increase in primary infection cycle, which starts in autumn, observed on the early sown plots of our trials. However, the effect of sowing date on secondary infections depended on the site. This might be related to the onset of secondary infection cycle: at those locations where the secondary infections start before winter, ear-

Table 8. Regression estimates for the effect of wheat cultural practices on disease progress model parameter c_2, which is associated to secondary eyespot development on wheat[a]

Cultural practice	Level	Experiment site			
		Le Rheu 92	Le Rheu 93[b]	La Verrière 92[b]	Chartres 92
Sowing date	early	0.0041	0.32	6.8×10^7	
	late	− 0.0041	− 0.32	-6.8×10^7	
Plant density	quantitative variable			-3.9×10^{5c}	
Shoots plant^{-2}	covariable	0.00169[d]		-7.4×10^{6e}	
Total nitrogen	high			-4.6×10^{6f}	
	low			4.6×10^{4f}	
Fertiliser form	NH_4	− 0.00058[g]			
	NH_4NO_3	0.00058[g]			
r^2		0.69	0.44	0.86	0

[a] Estimates are for model #4 shown in text; only effects significant at P = 0.05 are shown.
[b] A Box and Cox transformation was necessary. The transformation was $c_1{}^t = c_1{}^{(1-2.53/2)}$ at Le Rheu 93 and $c_1{}^t = c_1{}^{(1-1.51/2)}$ at La Verrière. Because of the negative exponent at La Verrière, the relationship between the estimates the levels of a given effect given for $c_1{}^t$ was therefore the inverse of that for c_1.
[c] The interaction between sowing date and plant density was significant; the number is the estimate of the correlation parameter on early sown plots.
[d] The interaction between sowing date and shoot number per plant was significant; the number is the estimate of the correlation parameter on late sown plots.
[e] The interaction between sowing date and shoot number per plant was significant; the number is the estimate of the correlation parameter on early sown plots.
[f] The interaction between sowing date and nitrogen rate was significant; the numbers are the estimated effects of nitrogen rate on early sown plots.
[g] The interaction between sowing date and fertiliser form was significant; the numbers are the estimated effects of nitrogen rate on early sown plots.

ly sowing would favour these infections (as at Le Rheu) because of the increase of the pre-winter infection period. However, the secondary cycle usually only starts in spring (Moreau et al., 1990); as late sowing means a longer post-winter period, secondary infections would be increased by late sowing, as observed at La Verrière. Similar results on the effects of sowing date on infection cycles have also been reported recently for take-all disease (Colbach et al., 1997). This analogy can be explained by the similarities of the epidemiologies of *P. herpotrichoides* and *Gaeumannomyces graminis* (Sacc.) von Arx et Olivier var. *tritici* (Walker), the causal pathogen of take-all. Both fungi survive on dead host residues in soil, begin their dissemination in autumn, do not spread from field to field (in contrast to air-borne diseases) and infect new host plants by inoculum produced by the surviving fungi on dead organic matter (resulting in the primary infection cycle) and by the necroses of living diseased plants (i.e. the secondary cycle).

The disease progress equations allow the complex effect of plant density to be analysed more closely. The impact of this factor on eyespot frequencies was illustrated either by a positive correlation of disease with plant density, or, more frequently, by a negative correlation with the shoot number per plant which was itself negatively correlated to plant density. Such observations have already been reported (Glynne, 1951; Salt, 1955; Huet, 1986; Groll and Luzi, 1991) and are explained by two mechanisms: (a) a high plant density means a shorter distance between the inoculum and its future host plant, and therefore a better probability of infection which is important for eyespot spreading by short-range spores (Glynne, 1953; Fitt and Lysandrou, 1984). Another explanation would be an increase in humidity in densely-sown crops as proposed by Groll and Luzi (1991). However, this effect would only be important when the canopy is closing. As the compensating effect of tillering usually leads to an equivalent number of shoots m^{-2}, whatever the initial sowing density, a variation in humidity does not seem the likely explanation for the effects of plant density; (b) a high plant density also means less tillering, while the emergence of new tillers is reported to push infected sheaths away from older shoots, thereby reducing infection by contact (Glynne, 1951; Salt, 1955; Huet, 1986). Furthermore, secondary tillers which are rare in high plant densities, also emerge later than the main

shoot and the primary tillers; they are therefore infected later and have less time to develop lesions. Because of this second mechanism, the covariable shoots per plant was more adequate for disease description than plant density itself. These two mechanisms, plant-inoculum distance and the effect of tiller emergence, also seem to influence the primary infection cycle, as the associated parameter c_1 was positively correlated with plant density. However, the situation must be different for secondary infections: plant density actually increased the secondary cycle at La Verrière and both at this location and at Le Rheu 92, the associated parameter c_2 was positively correlated with the number of shoots per plant. This second correlation is of course contradictory to the hypothesis of new tillers pushing away infected sheaths. This is not surprising, as tillering is mostly finished and the wheat canopy closed if the secondary cycle begins in spring. It is therefore relatively easy for the fungus to reach new host plants, which explains the lesser importance of the plant density factor for the secondary infections. In these circumstances, plants with a larger plant surface, caused by a higher shoot number per plant, would present a greater risk of being infected by eyespot, which would explain how the correlation between the shoot number and parameter c_2 was positive and not negative. This mechanism would also account for the positive correlation of shoot number per plant and eyespot frequency that was observed in Le Rheu 1992 at GS 80. These results can again be compared to those of Colbach et al. (1997) on take-all: the development of both diseases are increased at the beginning of wheat growth by a decrease in plant-inoculum distance (illustrated by a positive correlation between disease frequencies and plant densities) and, at later growth stages, by an increase in plant surface susceptible to the fungus (illustrated by the shoot or root number per plant). However, for take-all, both phenomena took place earlier and were more transient. Furthermore, for this disease, no event comparable to the removal of eyespot-infected sheaths by newly formed tillers was found.

For the other factors, i.e. nitrogen rates and fertiliser forms, the trials produced some new information. Previous reports on nitrogen rate are contradictory; eyespot increases (Meynard, 1985; Groll and Luzi, 1991) and decreases (Sieberasse and Fehrmann, 1987) as well as no influence of high nitrogen rate are reported (Shipton, 1972; Sekerkova et al., 1982; Steinbrenner and Höflich, 1984). With the help of the supplementary covariable shoot number per plant, two possible mechanisms with opposite effects (which would explain the previous contradictory results) were revealed in our trials: (a) an increase in nitrogen rate results in an increase in tillering, the latter decreasing eyespot incidence, so that high nitrogen would indirectly decrease disease; (b) an increase in nitrogen rate directly intensifies disease by an unknown mechanism. In contrast to other foot diseases such as foot rot (Ellen and Langerak, 1987), an increase in eyespot disease could not be related to plant nitrogen content; this content was higher on plots with high nitrogen rates at Le Rheu whereas eyespot was not influenced. However, plant tissue may be more susceptible to plant disease with high nitrogen rates, because of higher plant growth rates.

The mechanism related to tillering would only influence the primary cycle and the resulting early disease incidence (see above) whereas the second mechanism influenced both infection cycles; its effect was therefore not limited to an influence on the saprophytic and pre-infection stages of the fungus in the soil as the secondary cycle takes place outside this environment. The same methodology was used to study take-all (Colbach et al., 1997) and also concluded that two mechanisms existed with contradicting results, one influencing the fungus directly and one limiting its development by an indirect mechanism. There are, however, several important differences; only the primary infection cycle of take-all is stimulated by an increase in nitrogen, the inhibiting mechanism is not related to plant architecture, but to an expansion of antagonistic microflora in the case of nitrogen increase, and the disease-inhibiting mechanism is stronger than the stimulating one.

There are no previous reports on the effect of fertiliser form on eyespot. Results from our trials indicate a relationship between the decrease in disease on ammonium-fertilised plots and the secondary infection cycle, but give no further indications on the nature of this relationship (such as no indirect effect through reduced plant growth). Results with *G. graminis* showed that the effect of fertiliser form is greater than with eyespot and concerns both infection cycles (Colbach et al., 1997). Furthermore, the impact of fertiliser form on take-all is currently explained by a stimulation of antagonistic soil microflora (Smiley, 1978; Lucas and Collet, 1987; Sarniguet et al., 1992 a and b). However, microflora may be considerably less important for *P. herpotrichoides* in which only saprophytic survival takes place in soil whereas *G. graminis* also infects in this environment.

The multifactorial and multi-locational nature of our trials allowed new conclusions on the hierarchy

and interaction between various factors. Location was the dominant factor for the determination of disease build-up; if the location was unfavourable to disease development, eyespot incidence stayed low, even if all other factors were favourable, i.e. early sowing, high plant density and nitrogen rates. Sowing date was the dominant factor among cultural practices and its interaction with other practices was similar to that described previously; if sowing date was without an effect on eyespot, then no other practice was significant. Plant density, with the resulting shoot number per plant, was also a major cultural factor whereas nitrogen rate and fertiliser form were only minor factors.

The importance of primary and secondary infection cycles was determined only by statistical methods; no direct biological measures were done to confirm these results. Furthermore, this paper was limited to the study of disease incidence; the relationship between disease incidence and yield loss remains to be established. However, the percentage of diseased plants used as the output variable in this paper is not the most adequate variable to study subsequent yield loss. Despite this disadvantage, our results contribute to the understanding of the relative importance of crop management techniques to limit eyespot expression. The factorial experiments and modelling results show that environment and cultural practices have important effects on this disease. Cultural practices should not be considered individually, but crop management must be considered as a whole with respect to hierarchy and interactions. If the location is unfavourable, risk-inducing techniques such as early sowing will have little or no effect; on disease-prone sites, a late sowing, combined with a medium plant density, can limit disease risk sufficiently to allow high nitrogen rates thus a high yield objective.

Acknowledgements

We thank Dr. J.M. Meynard (INRA Grignon) for his help in improving this paper and B. Mille (INRA La Verrière) and H. Yvrard (Lycée agricole de Chartres) for conducting our field trials. This work was financed by the Grand-Duchy of Luxembourg and the Institut National de la Recherche Agronomique (France).

References

Box GEP, Hunter WG and Hunter JS (1978) Statistics for Experimenters: an introduction to design, data analysis and model building. Wiley, New York

Cavelier N, Lorée F and Prunier M (1992) Resistance of the eyespot fungus, *Pseudocercosporella herpotrichoides*, to DMI Fungicides. Proc Brighton Crop Protection Conf. 1992: 189–194

Cavelier N, Rousseau M and Le Page D (1987) Variabilité de *Pseudocercosporella herpotrichoides*, agent du piétin-verse des céréales; comportement *in vivo* de deux types d'isolats et d'une population en mélange. Z Pflanzenkr Pflanzenschutz 94: 590–599

Clarkson JDS (1981) Relationship between eyespot severity and yield loss in winter wheat. Plant Pathol 30: 125–131

Colbach N and Huet P (1995) Modelling the frequency and severity of root and foot diseases in winter wheat monocultures. Eur J Agron 4: 217–227

Colbach N, Lucas P and Cavelier N (1994) Influence des successions culturales sur les maladies du pied et des racines du blé d'hiver. Agronomie 14: 525–540

Colbach N, Lucas P and Meynard JM (1997) Influence of crop management on take-all development and infection cycles on winter wheat. Phytopathology 87: 26–32

Colbach N and Meynard JM (1995) Soil tillage and eyespot: influence of crop residue distribution on disease development and infection cycles. Eur J Plant Pathol 101: 601–611

Creighton NF, Cavelier N and Fitt BDL (1989) Pathogenicity to wheat and rye of *Pseudocercosporella herpotrichoides* isolates form France and the UK. Mycol Res 92: 13–17

Ellen J. and Langerak C.J. (1987) Effects of plant density and nitrogen fertilization in winter wheat (*Triticum aestivum* L.). 2. Incidence of *Gerlachia nivalis* and *Fusarium* spp. related to yield losses. Neth J Agric Sci 35: 155–162

Fitt BDL and Lysandrou M (1984) Studies on mechanism of splash dispersal of spores, using *Pseudocercosporella herpotrichoides* spores. Phytopathol Z 111: 323–331

Frahm J and Knapp A (1986) Ein einfaches Modell zur Optimierung von Fungizidbehandlungen gegen *Pseudocercosporella herpotrichoides*, in Weizen. Gesunde Pflanzen 38: 139–150

Glynne MD (1951) Effect of cultural treatments on wheat and on the incidence of eyespot lodging, take-all and weeds. Ann Appl Biol 38: 665–688

Glynne MD (1953) Production of spores by *Cercosporella herpotrichoides*. Trans Br Mycol Soc 36: 46–51

Glynne MD and Slope DB (1959) Effects of previous wheat crops, seed rate and nitrogen on eyespot, take-all, weeds and yields of two varieties of winter wheat. Ann appl Biol 47: 187–199

Groll U and Gutsche V (1989) Computergestütztes Prognoseverfahren für die Halmbruchkrankheit am Winterweizen (CERCOPROG). Ergebnisse der Erprobung 1986 bis 1988. Nachrichtenbl Pflanzenschutz DDR 43: 157–160

Groll U and Luzi K (1991) Untersuchungen zum Einfluß acker- und pflanzenbaulicher Faktoren auf den Halmbruchbefall an Wintergetreide. Archiv Phytopathol Pflanzensch 27: 459–470

Herman M (1985) Zhoknoceni vlievu zeleneho hnojeni a zaolani slamy na choroby pat stebel obilnin (An evaluation of the effect of green manuring on the rot in cereals). Sb Uvtiz Ochr Rostl 21: 81–87

Herman M (1986) Hodnoceni vekterych agrotechnickych zasalu z hlediska zdravotniho stavu psenice ozime (Evaluation of some agricultural measures in relation to health condition of winter wheat). Acta univ agric 3: 227–231

Higgins S and Fitt BDL (1985) Effects of water potential and temperature on the development of eyespot lesions in wheat. Ann Appl Biol 107: 1–9

Hollins TW and Scott PR (1980) Epidemiology of eyespot *Pseudocercosporella herpotrichoides* on winter wheat, with particular reference to the period of infection. Ann Appl Biol 5: 19–29

Huet P (1986) Influence du système de culture sur le piétin-verse du blé. In: Les rotations céréalières intensives. Dix années d'études concertées INRA-ONIC-ITCF 1973–1983 (pp. 95–111)INRA, Paris

Justes E, Mary B, Meynard JM, Machet JM and Thelier-Huche L (1994) Determination of a critical nitrogen dilution curve for winter wheat crops. Ann Bot 74: 397–407

Leroux P and Gredt M (1988) Caractérisation des souches de *Pseudocercosporella herpotrichoides*, agent du piétin-verse des céréales, résistantes à des substances antimitotiques et à des inhibiteurs de la biosynthèse des stérols. Agronomie 8: 719–729

Lucas P and Collet JM (1987) Influence de la fertilisation azotée sur la réceptivité d'un sol au piétin-échaudage, le développement de la maladie au champ et les populations de *Pseudomonas* fluorescents. Bull OEPP 18: 103–111

Maenhout CAAA (1975) Eyespot in winter wheat) effects of crop rotation and tillage, and the prediction of incidence. Bull OEPP 5: 407–413

Maraite H and Weyns J (1986) Evolution of MBC-resistance in *Pseudocercosporella herpotrichoides* on winter wheat and winter barley. Meded Fac Landbouwwet Rijksuniv Gent 51: 691–97

Mauler A and Fehrmann H (1987a) Erfassung der Anfälligkeit von Weizen gegenüber *Pseudocercosporella herpotrichoides*. I. Untersuchungen zur Pathogenität verschiedener Formen des Erregers. Z Pflanzenkr Pflanzenschutz 94: 630-636

Mauler A and Fehrmann H (1987b) Erfassung der Anhälligkeit von Weizen gegenüber *Pseudocercosporella herpotrichoides*. II. Zur Entwicklung eines Resistenztests an Jungpflanzen. Z Pflanzenkr Pflanzenschutz 94: 637–648

Meunier S (1984) Inventaire des maladies du pied chez le froment d'hiver en 1983 et incidence sur les composantes du rendement. Parasitica 40: 27–40

Meynard JM (1985) Construction d'itinéraires techniques pour la conduite du blé d'hiver. Thèse de docteur-ingénieur de l'INA-PG, 297 pp

Moreau JM, Van Schingen JC and Maraite H (1990) Epidémiologie de *Pseudocercosporella herpotrichoides* var. *acuformis* et var. *herpotrichoides* sur froment d'hiver, démonstration d'un cycle secondaire. Meded Fac Landbouwwet Rijksuniv Gent 55: 889–898

Nirenberg HI (1981) Differenzierung des Erregers der Halmbruchkrankheit. I. Morphologie. Z Pflanzenkr Pflanzenschutz 88: 241–248

Polley RW and Thomas MR (1991) Surveys of diseases of winter wheat in England and Wales (1976–1988). Ann Appl Biol 119: 1–20

Rapilly F, Laborie Y, Eschenbrenner P, Choisnel E and Lacroze F (1979) La prévision du piétin-verse sur blé d'hiver. Perspect agric 49: 30–40

Rémy JC and Hébert J (1977) Le devenir des engrais azotés dans le sol. CR Acad Agric Fr 63: 700–710

Salt GA (1955) Effects of nitrogen applied at different dates, and of other cultural treatments on eyespot, lodging and yield of winter wheat. Field experiment 1952. J agricSci, Cambridge46: 407–416

Sarniguet A, Lucas P and Lucas M (1992) Relationships between take-all, soil conduciveness to the disease, populations of fluorescent pseudomonads and nitrogen fertilizer. Plant Soil 145: 17–27

Sarniguet A, Lucas P and Lucas M (1992) Soil conduciveness to take-all of wheat: Influence of the nitrogen fertilizers on the structure of populations of fluorescents pseudomonads. Plant Soil 145: 29–36

SAS Institute Inc. (1989) SAS/STAT User's Guide, Version 6. Cary NC USA: SAS Institute Inc., Fourth Edition

Schaub R and Schlösser E (1982) Getreidefußkrankheitserreger. VI. Auswirkungen von *Pseudocercosporella herpotrichoides* und *Fusarium spp.* auf die Ertragskomponenten bei Winterweizen) Meded Fac Landbouwwet Rijksuniv Gent 47: 841–847

Schrödter H and Fehrmann H (1971a) Ökologische Untersuchungen zur Epidemiologie von*Cercosporella herpotrichoides*. II. Die Abhängigkeit des Infektionserfolges von einzelnen meteorologischen Faktoren. Phytophatol Z 71: 97–112

Schrödter H and Fehrmann H (1971b) Ökologische Untersuchungen zur Epidemiologie von *Cercosporella herpotrichoides*. III Die relative Bedeutung der meteorologischen Parameter und die komplexe Wirkung ihrer Konstellationen auf den Infektionserfolg. Phytophatol Z 71: 203–222

Schulz H, Bodker L, Nistrup Jorgensen L and Kristensen K (1990) Influence of different cultural practices on distribution and incidence of eyespot (*Pseudocercosporella herpotrichoides*) in winter rye and winter wheat. Tidsskr Planteavl 94: 211–221

Scott PR and Hollins TW (1974) Effects of eyespot on the yield of winter wheat. Ann Appl Biol 78: 269–279

Sekerkova M, Bojnansky V and Kandera J (1982) Vplyv dusikateho hnojemia na vyskyt huby *Cercosporella herpotrichoides* a na urody ozimnej psenice. Sb Uvtiz Ochr Rostl 18: 125–131

Shipton PJ (1972) Influence of stubble treatment and autumn application of nitrogen to stubbles on the subsequent incidence of take-all and eyespot. Plant Pathol 21: 147–155

Sieberasse G and Fehrmann H (1987) Ein erweitertes Modell zur praxisgerechten Bekämpfung des Erregers der Halmbruchkrankheit *Pseudocercosporella herpotrichoides* in Winterweizen. Z Pflanzenkr Pflanzenschutz 2: 137–149

Smiley RW (1978) Colonization of wheat roots by *Gaeumannomyces graminis* inhibited by specific soils, microorganisms and ammonium-nitrogen. Soil Biol Biochem 10: 175–179

Steinbrenner K and Höflich G (1984) Einfluß acker- und pflanzenbaulicher Maßnahmen auf den Befall des Getreides durch *Pseudocercosporella herpotrichoides* (Fron) Deighton und *Gaeumannomyces graminis* (Sacc.) Arx et Olivier. Arch Acker-Pflanzenbau, Bodenkd 20: 469–486

Steinbrenner K and Seidel D (1982) Komplexe Butzung acker. und pflanzenbaulicher Maßnahmen im Rahmen des Pflanzenschutzes. Nachrichtenbl Pflanzenschutz DDR 7: 137–140

Zadoks JC, Chang TT and Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14: 415–421