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Studies on *in vitro* growth and pathogenicity of European *Fusarium* fungi

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Abstract

The effect of temperature on the *in vitro* growth rates and pathogenicity of a European *Fusarium* collection consisting of isolates of *Fusarium graminearum, F. culmorum, F. avenaceum*, *F. poae* and *Microdochium nivale* was examined. Irrespective of geographic origin, the optimum temperature for the growth of *F. graminearum*, *F. culmorum* and *F. poae* was 25 °C, while that for *F. avenaceum* and *M. nivale* was 20 °C. In general, the growth rates of *F. graminearum*, *F. culmorum* and *F. poae* increased between 10 and 25 °C and those of *F. avenaceum* and *M. nivale* increased between 10 and 20 °C. Pathogenicity tests were carried out by examining the effect of the five species on the *in vitro* coleoptile growth rate of wheat seedlings (cv. Falstaff). Irrespective of geographic origin, the temperature at which *F. avenaceum*, *F. culmorum* and *F. graminearum* caused the greatest retardation in coleoptile growth ranges 20–25 °C (>89.3% reduction), whilst for *F. poae* and *M. nivale* it was 10–15 °C (>45.6% retardation), relative to uninoculated control seedlings. In general, *F. culmorum* and *F. graminearum* were the most pathogenic of the five species, causing at least a 69% reduction in coleoptile growth at 10, 15, 20 and 25 °C. General linear model analysis (GLIM) showed that species accounted for 51.3–63.4% of the variation in isolate growth and from 19.5% to 44.3% of the variation in *in vitro* pathogenicity. Country of origin contributed from 22.6% to 51.9% to growth rate variation and from 0.73% to 7.61% to pathogenicity variation. The only significant correlation between *in vitro* growth and pathogenicity was that observed for *M. nivale* at 15 °C ($r = -0.803$, $P < 0.05$).

Introduction

Fusarium head blight (FHB), (also known as ear blight or scab), *Fusarium* foot rot and *Fusarium* seedling blight of wheat are commonly caused by *Fusarium culmorum*, *F. avenaceum* (*Gibberella avenacea*), *F. poae*, *F. graminearum* (*Gibberella zeae*) and *Microdochium nivale* (fries) (*Monographella nivalis*), formerly known as *F. nivale* (Parry et al., 1995). FHB has been recorded in most cereal growing areas in the world including, Canada (Campbell and Lipps, 1998), the USA (Bai and Shaner, 1996), Russia (Levitin, 2000) and Europe (Parry et al., 1995). FHB has received significant attention in recent years because of the impact that infection may have on yield (Bai and Shaner, 1996; Doohan et al., 1999), grain quality (Liggitt et al., 1997), mycotoxin contamination of grain (Visconti et al., 2000) and the lack of preharvest fungicides for disease control (Doohan et al., 1999; Simpson et al., 2001).

Environmental factors such as temperature, rainfall and humidity have a significant effect on the incidence of *Fusarium* spp. and disease severity (Sutton, 1982; Vigier et al., 1997). *F. graminearum* is amongst the most pathogenic species of the *Fusaria* causing FHB (Bai and Shaner, 1996; Campbell and Lipps, 1998). This species predominates in warmer, humid areas of the world such as the USA and Canada (Vigier et al., 1997). FHB epidemics caused by this species have been associated with wet years (Sutton, 1982; Clear and Patrick, 2000), and it has been shown that *F. graminearum* from America and Canada

has an optimum *in vitro* growth temperature of 25 °C (Campbell and Lipps, 1998). *F. culmorum*, *F. poae* and *M. nivale* are more commonly found in the cooler maritime regions of Northwest Europe (Parry et al., 1995; Maurin et al., 1996). *In vitro*, the optimum temperature for growth of *F. culmorum* and *F. avenaceum* from the UK is between 20 and 25 °C (Pettitt et al., 1996). Of the five common casual organisms of FHB, *M. nivale* has been reported to be the fastest growing species at 5 °C, whilst its optimum growth occurred between 15 and 20 °C (Parry et al., 1994; Pettitt et al., 1996).

In planta, temperature and rainfall play an important role in the production and dispersal of inoculum, and in the infection of wheat heads and stem bases, and this subsequently determines the incidence and severity of disease (Sutton, 1982; Pettitt et al., 1996). *F. graminearum* inoculum is generally formed under warm, moist conditions; the optimum temperature for perithecial production was 29 °C (range 16–31 °C), and for ascospore production it ranged between 13 and 33 °C, with the optimum being 25–28 °C (Sutton, 1982). The optimum temperature for ascospore dispersal was 16 °C (range 13–22 °C), and for infection of the wheat head, colonization and appearance of symptoms it was 25 °C (range 20–30 °C). Wheat ears are most susceptible to infection by *F. culmorum*, *F. avenaceum* and *F. poae* at 25 °C while infection of ears by *M. nivale* was optimal at 15 °C (Polley et al., 1991). Wheat heads are most susceptible to infection by *Fusarium* spp. at the flowering stage (anthesis) (Sutton, 1982; Bai and Shaner, 1996). Severe epidemics of FHB can occur in seasons with below average temperature and above average rainfall at the anthesis stage (Parry et al., 1994; Clear and Patrick, 2000).

Temperature also plays a critical role in mycotoxicosis epidemiology. Jimenez et al. (1996) found that the temperature at which maximal zearalenone mycotoxin was produced varied for different *Fusarium* species and concluded that the production of mycotoxins by the different *Fusarium* species is differentially affected by environmental conditions such as temperature. Di Menna et al. (1991) also reported that the production of zearalenone is strongly dependent on incubation conditions, on the fungal species and also on the strain. Velluti et al. (2000) found that in the presence of *F. graminearum*, fumonisin B1 production by *F. moniliforme* (Sheldon) was enhanced at 25 °C but not at 15 °C. However, co-incubation with *F. moniliforme* did not affect the amount of zearalenone production by *F. graminearum*.

The objective of this research was to analyse a collection of European *Fusarium* isolates for their *in vitro* temperature sensitivity and pathogenicity, and to determine any relationships between temperature sensitivity, pathogenicity, country of origin and *Fusarium* species.

Materials and methods

Origin and maintenance of isolates

The 110 *Fusarium* isolates used in this study were obtained from four EU countries: Hungary, Ireland, Italy and the UK. All isolates were obtained from infected wheat seeds. The collection consisted of *F. graminearum* from Hungary (10), Ireland (2) and Italy (1); *F. culmorum* from Hungary (3), Italy (5) and the UK (10); *F. avenaceum* from Ireland (10) and Italy (3); *F. poae* from Hungary (10), Italy (6) and Ireland (27) and *M. nivale* from Italy (10), the UK (10) and Ireland (4) (Table 1). Isolates were stored in 10% (v/v) glycerol at −70 °C and prior to use subcultured onto potato dextrose agar (PDA) and incubated at 20 °C.

In vitro *growth of* Fusarium *isolates*

Temperature sensitivity was assessed by analysing the *in vitro* growth rate on PDA at 10, 15, 20, 25 and 30 °C. Fresh PDA plates were inoculated with mycelial plugs (7 mm dia), excised from 7-day-old PDA cultures and sealed with parafilm. Three cultures per isolate were placed at each of the following temperatures: 10, 15, 20, 25 and 30 °C. Colony radii were measured 3 and 4 days post-inoculation and used to calculate isolate growth rate (mm day^{-1}); the experiment was repeated once.

Pathogenicity of Fusarium *isolates towards wheat seedlings*

Thirty-nine of the 110 *Fusarium* isolates (Table 1) were chosen based on the results of the temperature sensitivity experiment, in which selection depended on proximity to the mean growth rate for each of the five species. The isolates selected represented three groups; Group 1: Average growth rates, Group 2: Lowest growth rates and Group 3: Highest growth rates (Table 1). Isolate *in vitro* pathogenicity towards wheat (cv. Falstaff, susceptibility rating of 6 on a scale of 1–10) was assessed using a modified method

Table 1. Species, origin and code of *Fusarium* isolates used in the *in vitro* growth and pathogenicity studies

Species	Country of origin	*In vitro* growth test[a]	*In vitro* pathogenicity test
F. avenaceum	Ireland	PO4	
F. avenaceum	Ireland	M7A	X[b]
F. avenaceum	Ireland	I113B	
F. avenaceum	Ireland	198A	
F. avenaceum	Ireland	I104A	
F. avenaceum	Ireland	I104C	
F. avenaceum	Ireland	O5B	X[d]
F. avenaceum	Ireland	I98C	
F. avenaceum	Ireland	I103A	X[d]
F. avenaceum	Ireland	F10	
F. graminearum	Ireland	L18C	X[d]
F. graminearum	Ireland	L6	X[c]
F. poae	Ireland	P2A	X[c]
F. poae	Ireland	I105	
F. poae	Ireland	I67B	
F. poae	Ireland	G07	X[c]
F. poae	Ireland	P4D	
F. poae	Ireland	I72A	
F. poae	Ireland	L11	
F. poae	Ireland	CC339B	X[d]
F. poae	Ireland	I113C	
F. poae	Ireland	I72C	
F. poae	Ireland	CC304A	
F. poae	Ireland	I113A	
F. poae	Ireland	M7C	
F. poae	Ireland	L1B	X[b]
F. poae	Ireland	C4	
F. poae	Ireland	MAIZE A	
F. poae	Ireland	CC359B	
F. poae	Ireland	O8	
F. poae	Ireland	07A	
F. poae	Ireland	I81A	
F. poae	Ireland	G6	
F. poae	Ireland	L18B	
F. poae	Ireland	I108C	
F. poae	Ireland	C05A	
F. poae	Ireland	G5	
F. poae	Ireland	I66	
M. nivale var. *majus*	Ireland	I96A	
M. nivale var. *majus*	Ireland	M7B	X[d]
M. nivale var. *majus*	Ireland	I67A	X[b]
M. nivale var. *majus*	Ireland	A2	X[c]
F. avenaceum	Italy	O66	X[c]
F. avenaceum	Italy	O67	X[d]
F. avenaceum	Italy	O68	X[b]
F. culmorum	Italy	O69	
F. culmorum	Italy	O70	
F. culmorum	Italy	O71	
F. culmorum	Italy	O72	X[b]
F. culmorum	Italy	O73	X[d]
F. graminearum	Italy	O74	X[b]
F. poae	Italy	2738	
F. poae	Italy	2739	
F. poae	Italy	2740	
F. poae	Italy	2741	X[d]
F. poae	Italy	2742	X[d]
F. poae	Italy	2743	X[d]
M. nivale	Italy	O75	
M. nivale	Italy	O76	
M. nivale	Italy	O7	
M. nivale	Italy	820	X[b]
M. nivale	Italy	821	
M. nivale	Italy	825	X[b]
M. nivale	Italy	2744	
M. nivale	Italy	2745	
M. nivale	Italy	2746	
M. nivale	Italy	2747	X[c]
F. culmorum	UK	FCF200	X[c]
F. culmorum	UK	FCG0/2	
F. culmorum	UK	124/3G	
F. culmorum	UK	FCFU36	
F. culmorum	UK	FC53	X[c]
F. culmorum	UK	124/6G	X[b]
F. culmorum	UK	FC70	
F. culmorum	UK	FCF95WW	
F. culmorum	UK	FCFU42	
F. culmorum	UK	FC302	
M. nivale var. *majus*	UK	SO54/1/M	
M. nivale var. *majus*	UK	2/2/M	
M. nivale var. *majus*	UK	SO48/7/M	
M. nivale var. *majus*	UK	44/3/M	
M. nivale var. *majus*	UK	SO53/3/M	
M. nivale var. *nivale*	UK	44/S/N	
M. nivale var. *nivale*	UK	SO20/1/N	
M. nivale var. *nivale*	UK	SO28/2/N	X[c]
M. nivale var. *nivale*	UK	SO48/1/N	X[b]
M. nivale var. *nivale*	UK	12/1/N	X[d]
F. culmorum	Hungary	HUCU1	X[d]
F. culmorum	Hungary	HUCU2	X[c]
F. culmorum	Hungary	HUCU3	X[b]
F. graminearum	Hungary	HUGR1	X[d]
F. graminearum	Hungary	HUGR2	X[c]
F. graminearum	Hungary	HUGR3	
F. graminearum	Hungary	HUGR4	X[b]
F. graminearum	Hungary	HUGR5	
F. graminearum	Hungary	HUGR6	
F. graminearum	Hungary	HUGR7	
F. graminearum	Hungary	HUGR8	
F. graminearum	Hungary	HUGR9	
F. graminearum	Hungary	HUGR10	
F. poae	Hungary	HUPO1	
F. poae	Hungary	HUPO2	

Table 1. (*Continued*)

Species	Country of origin	*In vitro* growth test[a]	*In vitro* pathogenicity test
F. poae	Hungary	HUPO3	
F. poae	Hungary	HUPO4	
F. poae	Hungary	HUPO5	
F. poae	Hungary	HUPO6	X[d]
F. poae	Hungary	HUPO7	X[d]
F. poae	Hungary	HUPO8	
F. poae	Hungary	HUPO9	X[b]
F. poae	Hungary	HUPO10	

[a]Isolates used the *in vitro* temperature sensitivity experiment. X – Isolates used in the *in vitro* pathogenicity experiment.
[b]Isolates with average growth rates.
[c]Isolates with lower growth rates than average.
[d]Isolates with higher growth rates than average.

of Mesterhazy (1983). Mycelium was produced by inoculating 50 ml of potato dextrose broth (PDB) with three mycelium plugs (7 mm dia) from a 7-day-old PDA culture. Following incubation in an orbital shaker running at 250 rpm at 20 °C, mycelium was harvested by centrifugation (5000*g*, 10 min), homogenised, and diluted to 13.3 mg mycelial homogenate ml^{-1} with 0.2% Tween 20 surfactant. Sterile Whatman No. 1 filter paper was placed on fresh PDA plates and 8 ml of the mycelium homogenate (or sterile PDB for control plates) positioned on the filter paper; a second sterile filter paper was placed on top of the inoculum. Wheat seed (cv. Falstaff) was surfaced-sterilised (in a 1% vv^{-1} solution of NaOCl) for 10 min, air dried, and 25 seeds placed on each PDA plate. Three plates per isolate were incubated at the following temperatures: 10, 15, 20, 25 and 30 °C. Coleoptile growth measured after 2, 3 and 4 days was used to determine the growth rate (mm day^{-1}), and results were expressed as percentage coleoptile growth rate retardation, relative to uninoculated control seedlings. The experiment was repeated once.

Statistical analysis

Normal data distribution was confirmed using Minitab (Minitab release 13©, 1994 Minitab incorporated, USA). The correlation coefficients between replicate experiments were determined using the Pearson Product Moment Correlation (Snedecor and Cochran, 1980). Analysis of variance incorporating Tukey's pairwise comparison at the 5% level of significance was performed using SPSS (SPSS Inc. Chicago, v8.02, 1989–1997). The contribution of species and county of origin to deviance in *in vitro* isolate growth rate and coleoptile growth retardation was assessed using general linear interactive model analysis (GLIM) (NAG Software, 1993). The relative contributions of species, country of origin and their interaction were determined by comparing three models (1) no explanatory variable, model 1 gave the general mean and no deviance; (2) species or country of origin as explanatory variable; (3) both species and country of origin. Model adequacy was measured by the percentage of deviance explained. Deviance was estimated according to a normal distribution of the error, and using identity as a link function. The term 'deviance' used in GLIM corresponds to the term 'variance' commonly used in other statistical tests.

Results

Influence of incubation temperature on growth rate of Fusarium *species*

Figure 1 depicts the growth of *F. poae* after 4 days incubation at 10, 15, 20, 25 and 30 °C (A–E). Irrespective of geographic origin, the optimum temperature for the growth of *F. culmorum*, *F. graminearum* and *F. poae* was 25 °C (Figure 2A) (mean growth rates = 8.2, 6.8 and 5.5 mm day^{-1}, respectively), while that for both *F. avenaceum* and *M. nivale* was 20 °C (Figure 2A) (mean growth rates = 3.0 and 7.4 mm day^{-1}, respectively). However, there was no significant difference between the 20 and 25 °C growth rates of either *F. culmorum* (mean growth rates = 8.0 and 8.2 mm day^{-1}, respectively) or *F. poae* isolates (mean growth rates = 5.3 and 5.5 mm day^{-1}, respectively).

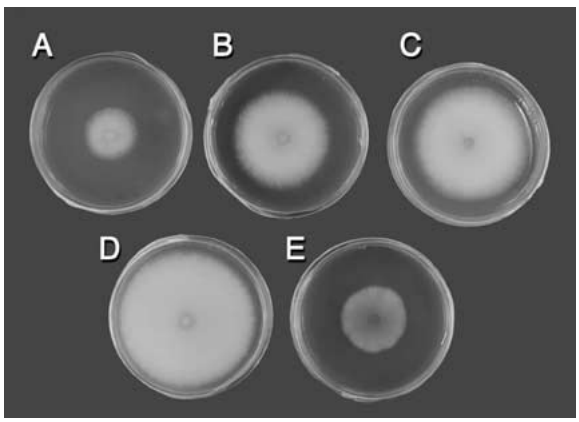

Figure 1. *In vitro* growth rate of *F. poae* (strain CC359B) at 10 (A), 15 (B), 20 (C), 25 (D) and 30 °C (E).

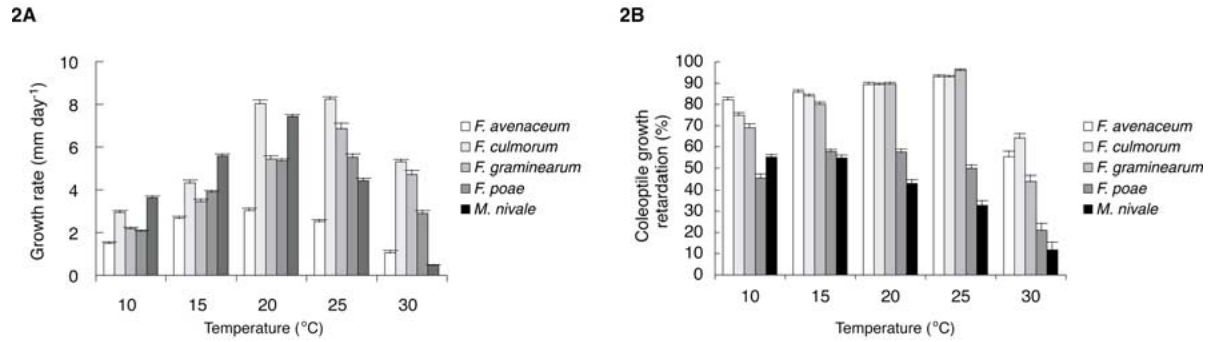

Figure 2. Influence of temperature on the *in vitro* growth rate (A) and pathogenicity (B) of five *Fusarium* species. Growth rate was assessed on PDA. Bars represent SEM.

Table 2. Contribution of species and country of origin to deviance in isolate growth rate, as assessed by generalised linear model analysis

Models		Deviance	Incubation temperature				
			10 °C	15 °C	20 °C	25 °C	30 °C
Model 1[a]		Total	92.75	166.68	408.71	586.71	536.67
		Explained	0%	0%	0%	0%	0%
Model 2[b]	Model 1 + species	Residual	36.27	77.55	149.59	285.44	204.51
		Explained	61%	54%	63%	51%	62%
	Model 1 + country	Residual	44.64	129.01	221.64	438.05	305.19
		Explained	52%	23%	46%	25%	43%
Model 3[c]	Model 1 + species & country	Residual	21.25	65.22	103.67	196.87	42.24
		Explained	77%	61%	75%	66%	92%

[a]Model 1 explained no variation.
[b]Model 2 determined % variation explained by species and country of origin.
[c]Model 3 determined % variation explained by combination of species and country of origin and their interaction.

There was a significant difference between the growth rates of all five species at 25 °C ($P < 0.05$), while at 20 °C, there was no significant difference between the growth rates of *F. graminearum* isolates and *F. poae* isolates ($P > 0.10$).

In general, *F. culmorum* had the fastest growth rate over the five incubation temperatures. The growth rates of *F. culmorum, F. graminearum* and *F. poae* increased between 10 and 25 °C and decreased between 25 and 30 °C. *F. graminearum* and *F. poae* had similar growth rates at 10, 15 and 20 °C ($P > 0.10$), but at 25 and 30 °C *F. graminearum* grew significantly better than *F. poae* ($P < 0.05$). Of the five species, *F. avenaceum* was the slowest-growing at 10, 15, 20 and 25 °C (mean growth rates ≤ 1.5 mm day^{-1}), while at 30 °C, *M. nivale* was the slowest-growing species (mean growth rate $= 0.5$ mm day^{-1}). The growth rates of *F. avenaceum* and *M. nivale* increased between 10 and 20 °C and decreased between 20 and 30 °C.

GLIM analysis showed that temperature accounted only for 1.4% deviance in the growth rate and GLIM analysis of individual temperature data explained from 51% to 63% (model 2) of the deviance in the growth rates of the isolates (Table 2). The incubation temperature at which species made the greatest contribution to the deviance in the growth rates of the isolates was 20 °C (63%).

Influence of country of origin on in vitro *growth of* Fusarium *species*

The growth rates of the 110 *Fusarium* isolates (Table 1) were analysed with respect to their country of origin (Figure 3A–E). The isolates of *F. avenaceum* from Ireland grew significantly faster than those from Italy at both 10 and 15 °C ($P < 0.05$) (Figure 3A), but in contrast, the Italian isolates grew significantly better at the higher temperature of 30 °C ($P < 0.05$). There were no significant differences between the growth rates of the *F. culmorum* isolates from Italy, Hungary and the UK at 15 and 25 °C ($P > 0.10$) (Figure 3B). However, at 10 °C, the UK isolates grew significantly

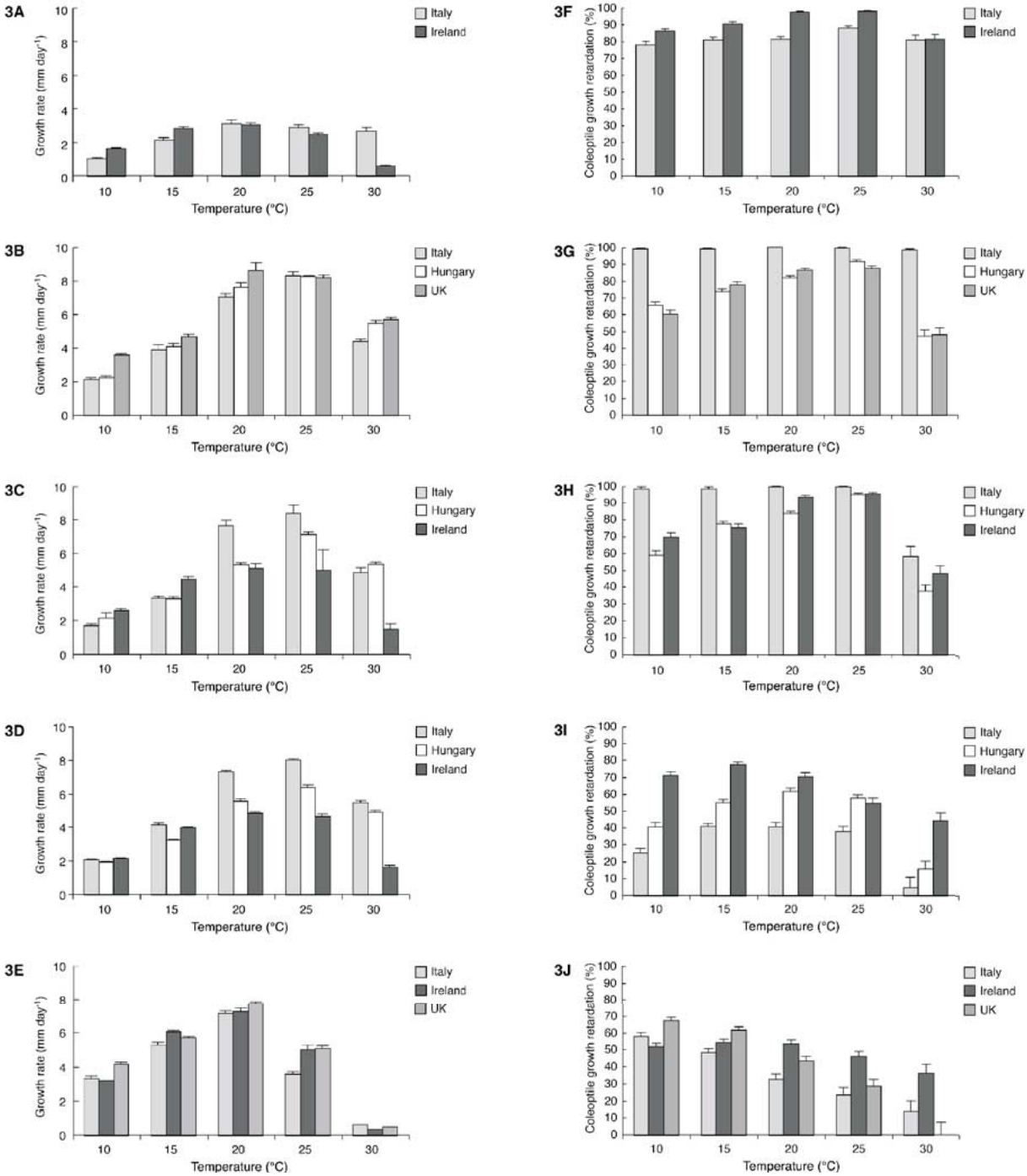

Figure 3. The effect of temperature on the *in vitro* growth rate and pathogenicity of *Fusarium* fungi, with respect to their country of origin. (A–E) represent the growth rate and (F–J) represent the percentage coleoptile retardation (relative to uninoculated control seedlings) attributed to *F. avenaceum* (A,F), *F. culmorum* (B,G), *F. graminearum* (C,H), *F. poae* (D,I) and *M. nivale* (E,J), respectively. Bars represent SEM.

faster than those from Italy or Hungary ($P < 0.05$) and at 30 °C, the Italian isolates grew significantly slower than those from both Hungary or the UK ($P < 0.05$). Of the *F. graminearum* isolates, the Italian isolates grew significantly better than those from Hungary or Ireland at 20 °C and better than the Irish isolates at 25 °C ($P < 0.05$) (Figure 3C). At 15 and 30 °C the Irish isolates grew significantly faster and significantly slower, respectively, compared to both the Italian and Hungarian isolates ($P < 0.05$) (Figure 3C). Analysis of the *F. poae* isolates originating from Italy, Hungary and Ireland (Table 1) showed that the Italian *F. poae* isolates grew faster than the Hungarian and Irish isolates at 20, 25 and 30 °C ($P < 0.05$) (Figure 3D). The Irish isolates grew slower than those from Italy or Hungary at 20, 25 and 30 °C ($P < 0.05$) (Figure 3D), and at 15 °C the Hungarian isolates grew significantly slower than both the Italian and the Irish isolates ($P < 0.05$). The *M. nivale* isolates originated from Italy, Ireland and the UK (Table 1) and at 15 °C, there was no significant difference in the growth rates of the *M. nivale* isolates from the UK and Italy or Ireland and at 20 °C there was no significant difference in the growth rates of the *M. nivale* isolates from Ireland and Italy or the UK ($P > 0.10$) (Figure 3E). At 10 °C the *M. nivale* isolates from the UK grew significantly faster than those from Italy or Ireland ($P < 0.05$), and at 25 °C the Italian isolates grew significantly slower than both the Irish and UK isolates ($P < 0.05$) (Figure 3E). Even though the growth rates of all *M. nivale* isolates were low at 30 °C, Italian isolates grew significantly faster than UK and Irish isolates ($P < 0.05$).

GLIM analysis was used to determine the contribution of country of origin to the variations in growth rates observed amongst *Fusarium* isolates (Table 2). Depending on the incubation temperature, country of origin explained from 23% (at 15 °C) to 52% (at 10 °C) of the deviation in the growth rates (model 2, Table 2). The combination of species and country of origin factors and their interaction (Table 2) explained >61% of the deviance in the growth rates of the isolates at all temperatures. The combination of both factors, species and country of origin explained up to 92% of the deviance in the growth rates of the isolates at 30 °C.

Pathogenicity of Fusarium *species towards wheat seedlings*

An *in vitro* Petri dish experiment was used to examine the effect of *F. avenaceum*, *F. culmorum*, *F. graminearum, F. poae* and *M. nivale* inoculations on the coleoptile growth rates of wheat seedlings (cv. Falstaff). Of the 110 *Fusarium* isolates, 39 were chosen for this study based on mean growth rate at the optimum incubation temperature. The coleoptile growth rates of uninoculated control seedlings were 0.6, 1.2, 2.3, 2.6 and 1.4 mm day^{-1} at 10, 15, 20, 25 and 30 °C, respectively. *F. avenaceum*, *F. culmorum* and *F. graminearum* were most pathogenic at 20–25 °C, *F. poae* at 15–20 °C and *M. nivale* at 10–15 °C (Figure 2B). At 20–25 °C, *F. avenaceum*, *F. culmorum* and *F. graminearum* caused >83.3% retardation in coleoptile growth rate, relative to uninoculated control seedlings. At 10–15 °C, *F. poae* and *M. nivale* caused >45.6% retardation in coleoptile growth rate.

Of the five *Fusarium* species, *F. graminearum* was the most pathogenic, causing a 96% reduction in coleoptile growth rate at 25 °C and at least a 44% reduction at the other four temperatures, relative to the uninoculated control seedlings. Figure 4 depicts the effect of *F. graminearum* (A) and *F. poae* (B) on coleoptile growth of wheat seedlings (cv. Falstaff) after 6 days incubation at 15 °C. *M. nivale* was the least pathogenic, causing at most a 55% reduction at 10 °C in coleoptile growth rate, relative to the uninoculated control seedlings. *F. avenaceum*, *F. culmorum* and *F. graminearum* were equally pathogenic at 20 and 25 °C, ($P > 0.10$) causing >89% growth rate retardation (Figure 2B). However, at 30 °C, *F. culmorum* was significantly more pathogenic than *F. avenaceum* and *F. graminearum,* causing a 64.2% reduction in coleoptile growth rate ($P < 0.05$). The coleoptile growth rates of the seedlings inoculated with *F. poae* and *M. nivale* were not significantly different at 15 and 30 °C, relative to the uninoculated control treatment ($P > 0.10$). At 20 and 25 °C, *F. poae* was more pathogenic than *M. nivale*, causing respective

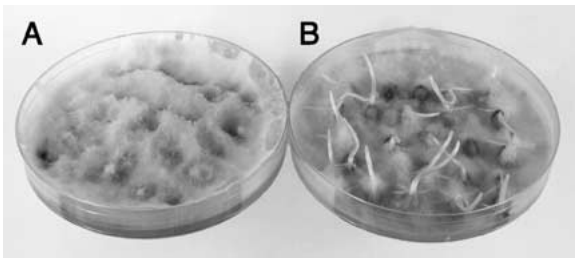

Figure 4. Effect of *F. graminearum* (strain 074) (A) and *F. poae* (strain 2742) (B) on coleoptile growth rate of wheat seedlings (cv. Falstaff).

Table 3. Contribution of species and isolate country of origin to deviance in isolate pathogenicity (coleoptile growth rate retardation) as assessed by generalised linear model analysis

Models		Deviance	Incubation temperature				
			10 °C	15 °C	20 °C	25 °C	30 °C
Model 1[a]		Total	32912	25051	41372	63777	88322
		Explained	0%	0%	0%	0%	0%
Model 2[b]	Model 1 + species	Residual	26492	17768	25608	35544	62563
		Explained	20%	29%	38%	44%	29%
	Model 1 + country	Residual	31670	24867	40222	61433	81598
		Explained	4%	0.7%	3%	4%	8%
Model 3[c]	Model 1 + species & country	Residual	18777	13645	22312	33626	50571
		Explained	43%	46%	46%	47%	43%

[a]Model 1 explained no variation.
[b]Model 2 determined % variation explained by species and country of origin.
[c]Model 3 determined % variation explained by combination of species and country of origin and their interaction.

reductions of 57.4% and 43% at 20 °C, and 49.8% and 32.6% at 25 °C, relative to the control seedlings ($P < 0.05$) (Figure 2B).

GLIM analysis showed that the incubation temperature at which species factor made the greatest contribution towards deviation in relative coleoptile growth (44%) was 25 °C (Table 3). Species accounted for 20%, 29%, 38% and 29% of relative coleoptile growth rate deviation at 10, 15, 20 and 30 °C, respectively.

Influence of country of origin on the pathogenicity of Fusarium *species*

The effect of *Fusarium* inoculation on coleoptile growth rates of wheat seedlings was analysed with respect to the country of origin of the isolates (Figure 3F–J). Of the *F. avenaceum* isolates, the Irish isolates were the most pathogenic ($P < 0.05$), causing at least 8% greater retardation in coleoptile growth rate (relative to the uninoculated control seedlings), than the Italian isolates at 10, 15, 20 and 25 °C (Figure 3F). Of the *F. culmorum* isolates, those originating from Italy were significantly more pathogenic than those from Hungary or the UK (Figure 3G), causing >98.3% retardation ($P < 0.05$) in coleoptile growth rate at 10–30 °C. There was no significant difference in the pathogenicity of *F. culmorum* isolates from Hungary and the UK at 10, 15 and 30 °C (mean coleoptile growth rates of 0.22, 0.30 and 0.82 mm day^{-1} for Hungarian isolates, and 0.26, 0.26 and 0.78 mm day^{-1} for the UK isolates, respectively) ($P > 0.10$). At 20 °C, the UK isolates were more pathogenic than the Hungarian isolates ($P < 0.05$), and at 25 °C the Hungarian isolates were more pathogenic than the UK isolates ($P < 0.05$), causing at least 4.6% and 4% more retardation in coleoptile growth rate, respectively. The Italian *F. graminearum* isolates were significantly more pathogenic than the Hungarian or Irish isolates at all temperatures except 30 °C ($P < 0.05$), causing at least 4.1% more growth retardation at 10–25 °C (Figure 3H). While the Hungarian *F. graminearum* isolates caused less growth rate retardation than the Irish isolates at all temperatures except 15 °C, only the differences observed between the Hungarian isolates and the Italian and Irish isolates at 10 and 20 °C were significant ($P < 0.05$) (Figure 3H); however at 30 °C there was a significant difference between the Hungarian and Italian isolates. Of the *F. poae* isolates, those originating from Ireland were significantly more pathogenic than those from Italy or Hungary at all temperatures except 25 °C ($P < 0.05$) (Figure 3I), causing at least 8.9% more growth rate retardation at 10, 15, 20 and 30 °C. At 25 °C, both the Irish and Hungarian isolates were equally pathogenic ($P > 0.10$), causing 54.4% and 57.4% coleoptile growth rate retardation, respectively. The Italian *F. poae* isolates were significantly less pathogenic than the Hungarian isolates at all temperatures except 30 °C ($P < 0.05$), causing at least 11.2% less growth rate retardation. Of the *M. nivale* isolates, the Irish isolates were significantly more pathogenic at 20, 25 and 30 °C ($P < 0.05$) (Figure 3J), causing at least 10.1% more growth retardation than the UK and Hungarian isolates (Figure 3J). At 10 °C the UK isolates were significantly more pathogenic ($P < 0.05$), causing at least 9.6% more growth rate retardation than both the Hungarian and Irish isolates. However at 15 °C there was no significant difference in the pathogenicity of the isolates from Ireland and Italy

or Hungary ($P > 0.10$), but there was a significant difference in the pathogenicity of the isolates from Italy and the UK ($P < 0.05$).

GLIM analysis showed that country of origin (model 2) explained less deviance in the relative coleoptile growth rate retardation than did species (0.7% at 15 °C, to 8% at 30 °C) (Table 3). The species and country of origin factors and their interaction explained from 43% (at 10 and 30 °C) to 47% (at 25 °C) of the deviation in the coleoptile growth rate (Table 3). The incubation temperature at which factors species and country of origin combined made the greatest contribution towards deviation in the growth rate of the isolates was 25 °C (47%). It appears from this analysis that species was the most significant factor affecting pathogenicity of the *Fusarium* isolates and had the most significant effect at 25 °C.

Correlation between in vitro *growth and seedling pathogenicity*

Any relationship which might exist between *in vitro* growth rate and pathogenicity of the *Fusarium* isolates was assessed using the Pearson Product Moment Correlation Coefficient. Of the 25 correlation coefficients calculated for the five *Fusarium* species (*F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* and *M. nivale*) at each of the five incubation temperatures (10, 15, 20, 25 and 30 °C), only one was statistically significant ($P < 0.05$). This was a significant negative correlation between the *in vitro* growth rates of *M. nivale* isolates and the relative coleoptile growth rate retardation caused at 15 °C ($r = -0.803$; $P < 0.05$).

Discussion

This research set out to determine temperature sensitivity of *in vitro* growth and pathogenicity in a collection of European *Fusarium* isolates. The method adopted for *in vitro* growth measurements has been used previously for *Fusarium* fungi (Clear and Patrick, 2000). *In vitro* pathogenicity was assessed by an adaptation of the seedling method devised by Mesterhazy (1983, 1984). Mesterhazy (1984) found that the seedling and head blight tests correlated well for *F. graminearum* pathogenicity scores and therefore pathogenicity of the *F. graminearum* inoculum could be predicted for head blight development via seedling tests. He concluded that the seedling test could eliminate the least pathogenic inocula from experimental work. The relationship between *in vitro* pathogenicity and FHB for other species is not known; this is currently being investigated (Brennan et al., unpubl. data).

The optimum *in vitro* growth temperatures for the five *Fusarium* species used in this research were in agreement with those obtained by previous researchers. For example, Campbell and Lipps (1998) reported that *F. graminearum* isolates from the USA had an optimum temperature for *in vitro* growth of 25 °C, and earlier, Cook and Christen (1976) reported that USA *F. graminearum* isolates grew best between 24 and 28 °C, and had slightly higher optimum growth temperatures if lower water potentials prevailed. Experiments conducted at 35 °C showed that *F. graminearum* did not grow at this temperature even after 30 days. Pettitt et al. (1996) also reported a variation in response to temperature of individual *Fusarium* species and *M. nivale* from the UK; they found that the optimum temperature for growth of *F. culmorum* and *F. avenaceum* was between 20 and 25 °C whilst *M. nivale* grew best at 20 °C but was significantly the fastest growing species at 5 °C. The pathogenicity experiment in the current work highlighted the variation in coleoptile retardation caused by the five *Fusarium* species at different temperatures. Overall, the greatest pathogenicity was achieved by *F. graminearum* at 20–25 °C, and the least by *M. nivale* at 30 °C, relative to uninoculated control seedlings. Our results also showed that *F. avenaceum*, *F. culmorum* and *F. graminearum* caused >44% coleoptile retardation at all temperatures whilst *F. poae* and *M. nivale* caused >32.6% at 10–25 °C, but >11.6% at 30 °C. However, in contrast to the current research, Ponchet (1966) reported that in a glasshouse study *M. nivale* was non-pathogenic on seedlings above 16 °C, but as in the current work found it was very aggressive at temperatures from 0 to 10 °C. Maurin et al. (1996) reported that *M. nivale* infection requires a lower incubation temperature (10–15 °C) than *F. graminearum* and *F. culmorum* (up to 25 °C) when variation in the resistance of whole winter wheat plants to infection by *M. nivale* was assessed in the glasshouse. Wang et al. (1984) also reported the influence of temperature on pathogenicity. They examined the pathogenicity of fungi associated with root rot of subterranean clover (*F. avenaceum*, *Pythium irregulare*, *Rhizoctonia solani*, *F. oxysporum* and *Phoma medicaginis*) at different temperatures (10, 15, 20 and 25 °C) and reported that while the fungi caused root rot over this range of temperatures, the most severe root rot occurred at 10 °C. However, the effect

of temperature varied with individual fungi and their combinations, in particular, combinations involving *P. irregulare*.

Analysis of our results showed that of the species and country of origin factors, species was the most significant contributing to the variation observed in both *in vitro* growth rates and seedling pathogenicity, irrespective of incubation temperature. *In vitro* isolates of *F. culmorum* grew fastest followed by *F. graminearum*, *M. nivale, F. poae* and *F. avenaceum.* In terms of seedling pathogenicity, isolates of *F. culmorum* caused the greatest coleoptile retardation followed by *F. avenaceum*, *F. graminearum*, *F. poae* and *M. nivale.* This was expected, as previously published research has shown that different *Fusarium* species vary in their growth and pathogenicity. For example, *in vitro* growth rates of *F. avenaceum*, *F. culmorum* and *M. nivale* isolates from the UK were compared and results showed that *M. nivale* had the lowest growth rate followed by *F. avenaceum*; the highest growth rate was exhibited by *F. culmorum* (Pettitt and Parry, 1996).

Irrespective of incubation temperature, country of origin also contributed, but to a lesser extent than species, to the *in vitro* growth and pathogenic variation observed amongst the isolates. Results showed that variation in the growth rate of the isolates due to country was very obvious at temperature extremes (10 and 30 °C). Irrespective of species, isolates from the UK and Ireland generally grew better at the lower temperatures (10–25 °C), while the Italian and Hungarian isolates performed better at the higher end of the temperature range (20–30 °C). This is probably due to prevailing growth conditions in the respective countries of origin and that *Fusarium* isolates are adapted to these specific conditions, forming temperature ecotypes (Pettitt and Parry, 1996). Thus, pathogenic *Fusarium* isolates originating from a particular country may not be able to cause the same levels of infection on wheat grown in a different climate, but may, over time, be able to adapt and regain former levels of pathogenicity.

The combined contribution of the species and country of origin factors and their interaction accounted for most deviation in *in vitro* isolate growth rates but less so for isolate pathogenicity. The remaining variation in growth and pathogenicity could be associated with other factors such as humidity, and in the case of the wheat seedlings, the host/pathogen interaction.

A significant negative correlation was obtained between isolate *in vitro* growth rates and seedling pathogenicity for *M. nivale* at 15 °C; this indicated that under these conditions, these isolates stimulated rather than inhibited coleoptile growth. The occurrence of both positive and negative relationships between growth and pathogenicity, and the temperature dependency of such relationships suggests that the *Fusarium*–host interaction is highly complex. In contrast to the current research, Walker et al. (2001) reported the existence of both direct and indirect relationships between *in vitro* growth and *in vivo* pathogenicity among *F. graminearum*, *F. culmorum* and *F. avenaceum* isolates. The *F. avenaceum* isolate exhibited the lowest *in vitro* growth rate and in the glasshouse had the lowest disease score; in contrast a *F. culmorum* isolate had the second lowest *in vitro* growth rate but caused the highest disease score. Bai and Shaner (1996) also reported a direct relationship between *in vitro* growth and *in vivo* pathogenicity of *F. graminearum* isolates. They found that *F. graminearum* isolates from China had the best growth rate in culture (incubated at room temperature) and when used for glasshouse inoculations (23–25 °C), these isolates also caused greatest FHB severity.

In conclusion, the current research has highlighted the importance of temperature as an environmental factor in the growth and seedling pathogenicity of *Fusarium* species. Results also suggest that within Europe, temperature ecotypes exist for the five *Fusarium* species causing FHB on wheat.

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