European Journal of Plant Pathology **108:** 573–583, 2002.

Variation in pathogenicity associated with the genetic diversity of *Fusarium graminearum*

J.P. Carter[1], H.N. Rezanoor[1], D. Holden[1], A.E. Desjardins[2], R.D. Plattner[2] and P. Nicholson[1]
[1]*John Innes Centre, Norwich Research Park, Colney Lane, Norwich, NR4 7UH, UK (Phone: +44 1603 450000; Fax: +44 1603 450045; E-mail: paul.nicholson@bbsrc.ac.uk);* [2]*Mycotoxin Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, US Department of Agriculture, Peoria, IL 61604, USA*

Accepted 15 May 2002

Key words: Fusarium head blight, SCAR, RAPD, chemotype

Abstract

We screened 188 isolates of *Fusarium graminearum*, which originated from northwest Europe, the USA and Nepal, for genetic diversity using a sequence-characterised amplified region polymorphism (SCAR). On the basis of this analysis, 42 of the 118 isolates were selected for random amplified polymorphic DNA (RAPD) analysis. Three groups were identified, two of which, A and B, contained the isolates from Nepal, and a third, group C, contained the isolates from Europe and the USA. In pathogenicity tests on wheat and maize seedlings, group C isolates were more pathogenic than the group A and B isolates. The isolates were assigned chemotypes based on their ability to produce the trichothecene mycotoxins nivalenol (NIV) and deoxynivalenol (DON). Isolates from group A were equally likely to produce NIV or DON while group B isolates produced predominantly NIV, and group C isolates produced predominantly DON. Within group A, isolates of the two chemotypes were equally pathogenic to wheat but isolates with the NIV chemotype were significantly more pathogenic to maize. The results confirm that distinct genetic groups exist within *F. graminearum* and demonstrate that these groups have different biological properties, especially with respect to their pathogenicity to two of the most economically important hosts of this pathogen.

Introduction

Three of the world's most important crops, wheat, rice and maize, are susceptible to a number of *Fusarium* species including *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein) Petch). The fungus can infect many plant parts during the life cycle of the host and thus cause a range of diseases. *F. graminearum* can cause seedling blight, brown foot rot, and scab (also known as ear or head blight) of wheat, and both stem rot and ear rot of maize (Francis and Burgess, 1975; Miedaner and Schilling, 1996; Ou, 1985; Parry et al., 1995; Vigier et al., 1997).

A number of molecular techniques have been used to resolve difficulties with the identification of *F. graminearum* and to provide information on its population structure. A combination of morphological and molecular data allowed the division of *F. graminearum* into group 1 and group 2 isolates and the subsequent redesignation of group 1 *F. graminearum* as a separate species *F. pseudograminearum* (Aoki and O'Donnell, 1999). Species-specific PCR primers have been designed to differentiate *F. graminearum* isolates from those of related *Fusarium* species (Nicholson et al., 1998). Although this fungus has been isolated in all major agricultural regions of the world, little is known about the population structure of *F. graminearum* isolates from different geographical areas and whether these differences are related to pathogenicity or any other trait. Genetic variation within *F. graminearum* has been detected by random amplified polymorphic DNA

(RAPD) analysis. Although isolates from different continents have been examined (Schilling et al., 1994), most analyses have compared isolates from closer geographical sites, e.g., within Europe (Schilling et al., 1997), Canada (Dusabenyagasani et al., 1999), Nepal (Carter et al., 2000) and North Carolina, USA (Walker et al., 2001). While isolates from two regions (Ontario and Quebec) in Canada were considered to form a single population pool (Dusabenyagasani et al., 1999), two distinct groups were identified among Nepalese isolates (Carter et al., 2000). The latter study also characterised isolates using a sequence-characterised amplified region (SCAR). This revealed that all isolates of one group (termed B) possessed a single SCAR polymorphism while those of the second group (group A) had one of five different SCAR polymorphisms (Carter et al., 2000). The relationship among isolates from different geographic regions remains unclear. DNA sequence analysis has been used to compare *F. graminearum* isolates from around the world and a scheme dividing the species into seven biogeographically structured lineages was proposed (O'Donnell et al., 2000). The seven lineages and their proposed geographical origins, are, however, not fully supported by an analysis of RAPDs or of restriction fragment length polymorphisms of the intergenic spacers in the ribosomal RNA gene cluster (IGS RFLP) of isolates from Nepal (Carter et al., 2000), even though three isolates were common to both studies. Both the DNA sequence (O'Donnell et al., 2000) and RAPD (Carter et al., 2000) analysis suggest the existence of genetically distinct groups within *F. graminearum*.

Isolates of *F. graminearum* vary in their pathogenicity towards different hosts. The pathogenicity of Chinese and USA isolates has been tested on wheat, and in a comparison of six isolates, one Chinese isolate caused significantly greater disease symptoms than did any of the four isolates from the USA (Bai and Shaner, 1996). In a study of pathogenicity towards rye, the genetic variation among isolates derived from three continents, America, Australia and Europe, was greater than that obtained for strains from populations of *F. graminearum* from individual sites in Europe (Miedaner et al., 2000). The individual field populations contained 60% of the total variation observed, indicating that a high degree of variation in pathogenicity was present at each site. No relationship was established between pathogenicity and population although one group identified by RAPD analysis was found to be less frequently isolated from maize than from either rice or wheat (Carter et al., 2000).

Fusarium species can produce a range of mycotoxins, including trichothecenes, a family of sesquiterpene epoxides, (Joffe, 1986). Trichothecenes inhibit protein synthesis (Casale and Hart, 1988; Cundliffe et al., 1974; Ehrlich and Daigle, 1985), induce MAP kinases associated with apoptosis (Shifrin and Anderson, 1999) and have been associated with a wide range of human and animal diseases including anaemia, immunosuppression, haemorrhage and emesis (Marasas et al., 1984). *F. graminearum* can produce a range of 8-keto-trichothecenes, including 4-acetylnivalenol (4-AcNIV), nivalenol (NIV), deoxynivalenol (DON) and other acetylated derivatives. The loss of trichothecene production does not affect the ability of an isolate to infect wheat or maize, but it does affect the progression of an infection (Desjardins et al., 1996; Harris et al., 1999; Proctor et al., 1995). This suggests that trichothecenes may be factors that affect the progress of infections on both wheat and maize rather than pathogenicity factors controlling the ability for infection to occur. Chemotypes, based on the dominant trichothecenes produced, have been described for *F. graminearum* (Ichinoe et al., 1983; Miller et al., 1991), but no relationship has been established between chemotype and pathogenicity to barley, wheat, triticale, rye or maize (Logrieco et al., 1990; Perkowski et al., 1997) or to genetically distinguishable populations (O'Donnell et al., 2000). The lack of a relationship between chemotype and pathogenicity may mean that both the type and the amount of toxin produced affect pathogenicity (Atanassov et al., 1994). Chemotype variation is, however, important in determining the suitability of methods used to analyse the toxin content of human and animal feedstuffs.

The present work was undertaken to determine whether isolates of *F. graminearum* from Europe and USA were similar to either of the groups previously identified from Nepal (Carter et al., 2000). Further experiments were carried out to determine whether genetically distinguishable populations of *F. graminearum* differ in their pathogenicity. The genetic diversity within a collection of isolates of *F. graminearum* from the USA, Europe and Nepal was compared using SCAR and RAPD profiles. The pathogenicity of isolates from different genetic groups was then measured on both wheat and maize. In addition, the relationship between genetic diversity, chemotype and the host of origin were examined within one of the genetic groups which contained isolates from three hosts of origin and both DON and NIV chemotypes.

Materials and methods

Origin and maintenance of fungal strains

Isolates of *F. graminearum* from Germany and France were isolated in 1992 except for G5 (1997) while those from the UK were isolated in 1998. All isolates from the USA were obtained from farms across Illinois in 1998. Isolates from Nepal were isolated in 1997 except for MK7, ML4, ML6, ML13 and ML15; these were isolated in 1993. Cultures were maintained at 15 °C on potato dextrose agar (PDA) (DIFCO, West Molesey, Surrey, UK) containing streptomycin sulphate (50 mg l^{-1}) and penicillin-G (50 mg l^{-1}). For long-term preservation, mycelium was stored in vapour-phase liquid nitrogen vessels.

SCAR analysis

DNA was extracted from the mycelium of 4–5-day-old cultures of 118 isolates of *F. graminearum*; 28 from the USA, 25 from northwest Europe (France, Germany, and the UK) and 65 from Nepal, grown in potato dextrose broth (DIFCO). The mycelium was freeze-dried, ground to a fine powder, and the DNA extracted using the CTAB method of Nicholson et al. (1996). PCR amplification, using primer pair Fg16F/R (Nicholson et al., 1998), was carried out as described in Carter et al. (2000) and the products were separated by electrophoresis through a 2% agarose gel, stained with ethidium bromide and viewed under UV light. The size of PCR products was estimated by comparison with known DNA standards. Before sequencing, PCR products were purified using Qiaquick PCR purification spun columns (Qiagen Ltd, Crawley, West Sussex, UK) according to the manufacturer's instructions and sequenced in both directions using the ABI PRISM Big Dye terminator cycle sequencing kit (Perkin-Elmer, Warrington, Cheshire, UK) primed with either Fg16F or Fg16R (Nicholson et al., 1998). Sequencing reactions were run on an ABI PRISM DNA sequencer (Perkin-Elmer).

RAPD assays

Forty-two isolates were selected for RAPD analysis on the basis of their area of origin and SCAR type. The assay was carried out as described in Carter et al. (2000) using seven oligonucleotide primers OP-T01, OP-T04, OP-T06, OP-T16, OP-U13, OP-U15 and OP-U19 from kits OPT and OPU (Operon Technologies Inc, Alameda, CA). The data were analysed as described in Carter et al. (2000) using software contained in RAPDist (version 1.04 [http://life.anu.edu/molecular/software/rapd.htm]) and PHYLIP, (version 3.572; [http://evolution.genetics.washington.edu/phylip.html]). A programme to produce bootstrapped datasets was kindly supplied by John Armstrong (Australian National University, Canberra, Australia).

Pathogenicity tests

The 42 isolates used in the RAPD analysis were tested for the ability to infect wheat (cv. Chinese Spring) and maize (cv. F2) seedlings in a stem-base assay modified from that described by Simpson et al. (2000). Wheat and maize seed was germinated at 5 and 24 °C, respectively before five wheat or four maize seeds were planted into 250-ml pots containing a peat/sand mixture. The pots were placed in covered propagators at 20 °C with 16-h light/day and kept moist. Emergent seedlings were inoculated by pipetting 200 or 400 µl of mycelial macerate into a 0.5 or 0.9 cm internal diameter plastic collar placed around the stem of the wheat and maize seedlings respectively. The mycelial macerate was prepared by homogenising mycelium from PDA Petri plates, inoculated at the same time for each isolate and harvested before the hyphae had reached the edge of the plate, in distilled water (3 cm^2 mycelium/ml water). Four days after inoculation the covers were removed from the propagators and a further 17 days later the plants were scored on a 0–9 scale based on Simpson et al. (2000): 0, symptomless; 1, coleoptile slightly necrotic; 2, coleoptile severely necrotic; 3, coleoptile necrotic and first leaf slightly necrotic; 4, coleoptile and first leaf severely necrotic; 5, coleoptile and first leaf necrotic, second leaf slightly necrotic; 6, coleoptile, first leaf and second leaf severely necrotic; 7, coleoptile, first leaf and second leaf severely necrotic, third leaf slightly necrotic; 8, coleoptile, first, second and third leaves severely necrotic; 9, coleoptile, first, second, third and fourth leaf necrotic or whole seedling necrotic. The test was carried out twice with each experiment containing five pots of wheat seedlings and five pots of maize seedlings inoculated with each fungal isolate. The disease rating was calculated as a mean for the seedlings in each pot (mean of 5 seedlings per pot for wheat and 3.5 for maize). Data were tested for normal

distribution and equal variance before analysis using the accumulated ANOVA option under the general linear regression menu in Genstat 5, 4th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, Hertfordshire, UK). When ANOVA indicated a significant difference between treatments ($P < 0.05$) the treatments were compared using the Students' t-test available from the same menu.

Chemotype analysis

The ability of the isolates to produce NIV and DON was assessed in cultures grown for 4 weeks on autoclaved rice. The culture material was extracted with 86 : 15 (vol/vol) acetonitrile : water (5 ml g^{-1}), and 100 µl of extract was evaporated to dryness under nitrogen at 70 °C. Trimethylsilyl (Tms) derivatives were made by the addition of 100 µl Tms reagent (Tri-sil, Pierce Co., Rockford, IL, USA), heating at 70 °C for 1 h before the addition of 900 µl iso-octane and 1 µl was analysed by gas chromatography/mass spectrometry in the negative Cl mode. DON, NIV, 3-AcDON, 15-Ac DON and 4-AcNIV were identified by their retention time and by their mass spectra in comparison with authentic standards.

Results

SCAR analysis

Six different PCR products were identified by agarose gel electrophoresis after amplification by primer pair Fg16F/R, with each isolate yielding a single product (Table 1). All 28 isolates from the USA, 24 of the 25 isolates from Europe and three Nepalese isolates produced type 1 (0.42 kb) SCAR products. The remaining European isolate, D2, was the only isolate to yield a type 6 (0.40 kb) product. Nepalese isolates yielded four additional products, type 2 (0.51 kb), type 3 (0.54 kb), type 4 (0.58 kb) and type 5 (0.52 kb). The size variation in the PCR products is due to variation in the regions between nucleotides 79 and 168 and nucleotides 440 and 526, with only seven nucleotide positions out of the remaining 380 showing variation (Figure 1). The variation between nucleotide position 79 and 168 could be accounted for by differing numbers of a 30 nucleotide repeated motif between nucleotide positions 169 and 199. The shortest product, type 6, had only a single copy of this motif, whereas the longest product, type 4, had three additional copies of the repeat and type 3, two additional copies. The type 2 and type 5 products had the same sequence length with one additional, but different, repeat of the 30 nucleotide motif. The type 1 products from isolates G1 and G2 from Europe and isolate USA1 from the USA were identical in sequence and differed by three nucleotides from the type 1 product yielded by RL1 from Nepal. The type 1 products had two copies of the repeated motif but a deletion between nucleotides 440 and 526. D2, the only European isolate not to yield a type 1 product, shared this deletion between nucleotide positions 440 and 526 but contained only a single copy of the repeated motif.

Table 1. The distribution of the SCAR polymorphisms amongst *F. graminearum* isolates from Europe, Nepal and the USA

Geographical origin of isolates	SCAR polymorphism[a]						Total
	1	2	3	4	5	6	
Europe	24	0	0	0	0	1	25
Nepal	3	23	30	6	3	0	65
USA	28	0	0	0	0	0	28
							118

[a]The SCAR polymorphisms had the following apparent molecular weights after electrophoresis: type 1, 0.42 kb; type 2, 0.51 kb; type 3, 0.54 kb; type 4, 0.58 kb; type 5, 0.52 kb; and type 6, 0.40 kb. The sequences of the different products are shown in Figure 1.

RAPD analysis

We analysed RAPD patterns generated by seven primers with 42 strains (Table 2). We scored 105 bands, 18 of which were common to all isolates, and five of which were unique to individual isolates. Only two of the isolates, ML11 and ML12, both isolated from maize in Nepal, had identical profiles. A dendrogram constructed using the RAPD data (Figure 2) showed that the isolates divided into three groups. Group A, supported by a bootstrap value of 82%, contained 16 of the Nepalese isolates, and group B, supported by a bootstrap value of 100%, contained the remaining 6 Nepalese isolates. Group C, supported by a bootstrap value of 100%, contained all of the American and European isolates. All of the isolates within RAPD group B yielded a type 2 SCAR product, and all of the isolates within RAPD group C yielded a type 1 SCAR product with the exception of isolate D2 which yielded a type 6 product. RAPD group A, in contrast, contained

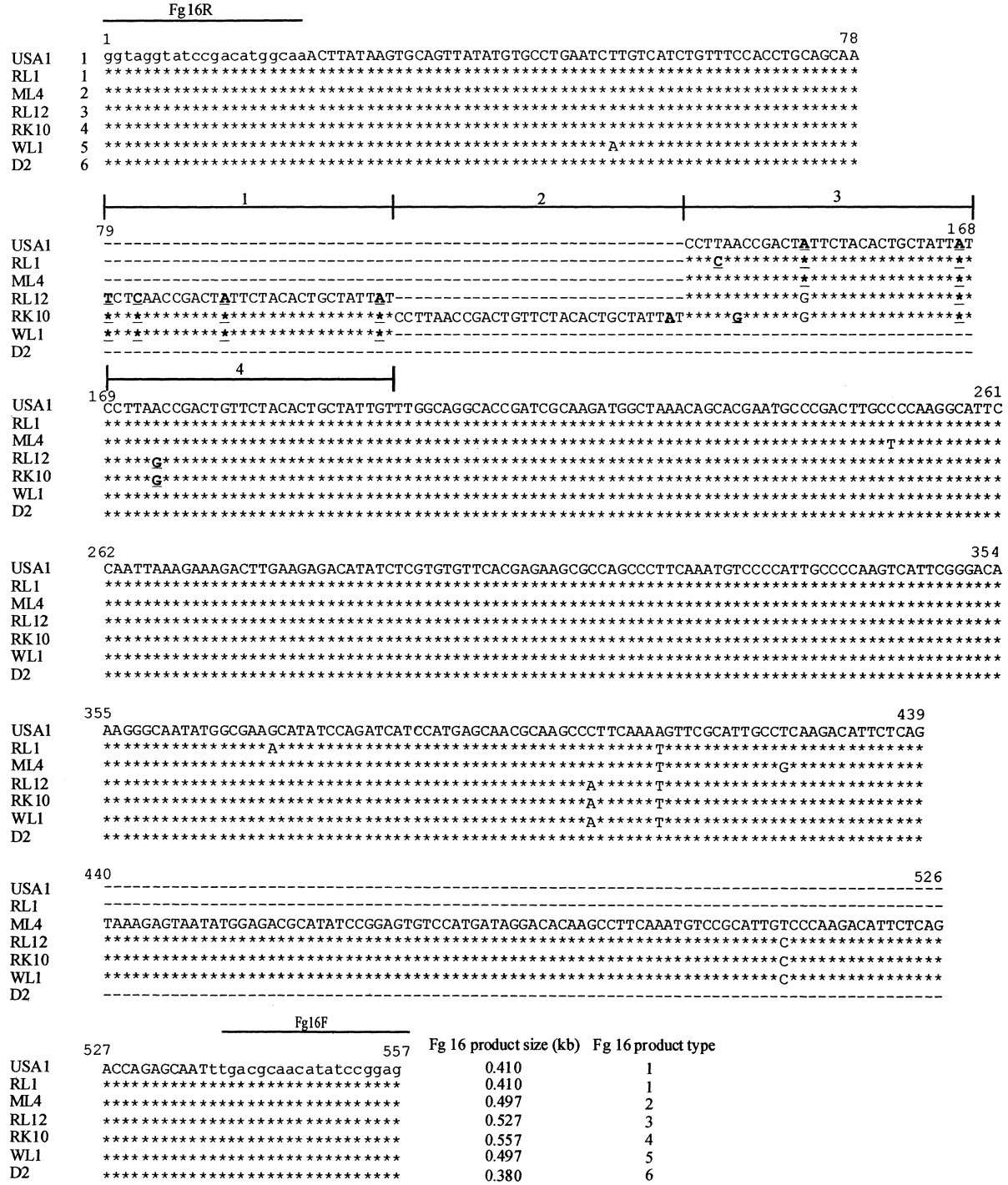

Figure 1. Nucleotide sequence alignment of the six Fg16 PCR products from isolates of *F. graminearum*. Nucleotides 79–168 relate to the region containing variable numbers of the 30 nucleotide sequence in the different sequences (1, 2 and 3) and which are similar to the sequence 169–199 (marked 4). The sequence of Fg16 product type 1 is shown with ∗ indicating sequence identity and – an introduced gap. Underlined nucleotides in regions 1, 2, 3 and 4 indicate variation from the Fg16 product type 1 sequence from area 4.

Table 2. The origin, SCAR and chemotype of *F. graminearum* isolates used in the RAPD and pathogenicity analysis

Isolate	Origin		SCAR[a]	Chemotype[b]
	Country	Host		
D1, D3, D4, D5, D6	Germany	Wheat	1	DON
D2	Germany	Wheat	6	DON
G1, G3, G4, G5	France	Wheat	1	DON
G2	France	Wheat	1	NIV
MK7, MK8, ML4, ML13	Nepal	Maize	2	NIV
MK9, ML15	Nepal	Maize	3	DON
ML6	Nepal	Maize	2	NC
ML11, ML12	Nepal	Maize	3	NIV
RK2	Nepal	Rice	3	NC
RK5	Nepal	Rice	4	DON
RK10	Nepal	Rice	4	NIV
RL1, RL2	Nepal	Rice	1	DON
RL12	Nepal	Rice	3	NIV
UK1, UK2, UK3	UK	Wheat	1	DON
USA1, USA2, USA3, USA4, USA5, USA6	USA	Wheat	1	DON
WK1, WL2	Nepal	Wheat	3	DON
WK5	Nepal	Wheat	2	NIV
WL1	Nepal	Wheat	5	NIV
WL9, WL11, WL12	Nepal	Wheat	3	NC
WL12	Nepal	Wheat	3	—

[a]The Fg16 product types had the following apparent molecular weights after electrophoresis: type 1, 0.42 kb; type 2, 0.51 kb; type 3, 0.54 kb; type 4, 0.58 kb; type 5, 0.52 kb; and type 6, 0.40 kb. The sequences of the different products are shown in Figure 1.

[b]DON, only DON detected; NIV, NIV detected together with low levels of DON (less than 5% of the level of NIV); NC, not classified (toxin production below detection limit); —, isolate not tested.

isolates that yielded a range of SCAR products, including type 1, 3, 4 and 5. Both of the isolates within group A that yielded type 1 products were grouped together with 100% bootstrap support; the long branch length between them and the other isolates within group A indicates that they may represent a further, fourth, RAPD group.

Pathogenicity assays

No significant interactions ($P > 0.1$) were detected between the block or test factors and isolate so the mean disease score for each isolate on each host was used in all subsequent analysis. The pathogenicity of an isolate to wheat was positively correlated with its pathogenicity to maize ($r = 0.8$). The mean pathogenicity score of 3.6 for RAPD group C isolates on wheat was higher than the scores for RAPD groups A and B, 2.9 and 2.4, respectively. Similarly the mean pathogenicity score of 6.9 for RAPD group C isolates was higher than the mean pathogenicity score of 4.1 for RAPD groups A and B isolates on maize. Analysis of variance (Table 3) demonstrated that the effect of RAPD group was highly significant for pathogenicity to both maize ($P = 0.004$) and wheat ($P = 0.005$). RAPD group C was significantly more pathogenic to both wheat and maize than RAPD groups A ($P = 0.013$ and $P = 0.002$) and B ($P = 0.005$ and $P = 0.02$) (Figure 3). There was no significant difference between the pathogenicity of RAPD groups A and B towards either wheat or maize ($P = 0.294$ and 0.972, respectively).

Chemotype

Of the 40 isolates tested for toxin production, 26 produced DON alone and 10 produced NIV plus trace levels of DON (less than 5% of the amount of NIV produced) (Table 2 and Figure 2). Toxin levels of between 10 and 100 $\mu g\, g^{-1}$ dry weight original culture material were detected for these 36 isolates. Isolates ML6, RK2, WL9 and WL11 produced no detectable, or only trace levels compared to the detection limit of 50 $ng\, g^{-1}$ dry weight original material, of both toxins, and could not be placed in a chemotype class. NIV producers also produced 4-AcNIV at concentrations that ranged from 20% to 200% of the level of NIV that was detected. All DON producers also accumulated AcDON (principally 3-AcDON but occasionally traces of 15-AcDON were also detected) but the level of AcDON detected was never greater than 5% of the level of DON measured. A χ^2 test showed that isolates within RAPD group A were equally likely to produce NIV or DON ($P = 0.58$). An analysis of variance of the pathogenicity data for the group A isolates indicated that chemotype (NIV, DON, or not classified) was not significantly associated with pathogenicity to wheat ($P = 0.195$) but was associated with pathogenicity to maize ($P = 0.005$) (Table 4). Isolates of RAPD group A that had a NIV chemotype were significantly more aggressive towards maize seedlings than isolates with a DON chemotype ($P = 0.004$) or isolates which had not been classified ($P = 0.003$)

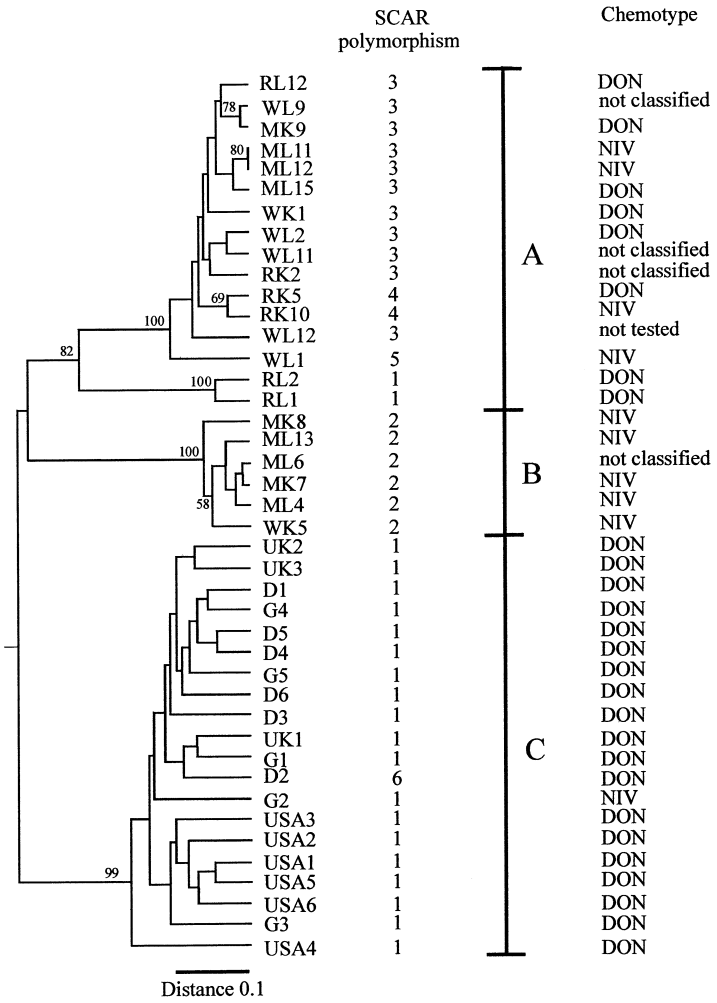

Figure 2. Relationship between the *F. graminearum* isolates based on RAPD analysis. The number after the isolate name refers to the Fg16 polymorphic PCR product yielded by the isolate. Production of the toxins nivalenol (NIV) and deoxynivalenol (DON) was assessed after the growth of isolates in rice culture and analysis by GC/MS. Dendrograms were produced after data were converted to a distance matrix using algorithm number 2 (Jaccard) of RAPDist version 1.04 (2). The distance matrices were used to construct the dendrograms by the unweighted pair group method with arithmetic mean (UPGMA) using the Neighbour programme contained in PHYLIP, version 3.572 (12). Bootstrapped datasets (500 replicates) were produced using a programme supplied by John Armstrong (ANU Australia) and analysed using the Neighbour and Consense programmes contained in PHYLIP. Bootstrap values greater than 50% are shown.

Table 3. Analysis of variance for the pathogenicity of RAPD groups within *F. graminearum* towards wheat and maize

	Wheat				Maize			
	d.f.[a]	m.s.[b]	v.r.[c]	*F*. pr.[d]	d.f.[a]	m.s.[b]	v.r.[c]	*F*. pr.[d]
RAPD group	2	4.0887	5.98	0.005	2	41.484	6.55	0.004
Residual	39	0.6836			39	6.331		
Total	41	0.8497			41	8.046		

[a]Degrees of freedom; [b]mean square; [c]*F* ratio; [d]*P* value from *F* distribution.

(Figure 4). Isolates of RAPD group A with DON chemotypes did not differ significantly in pathogenicity towards maize seedlings from the unclassified isolates. The isolates placed in group B, all of which had yielded a type 2 Fg16 product, produced NIV (five isolates) apart from ML6 which produced only very low levels of both toxins. All of the isolates from the USA and Europe, (RAPD group C and type 1 or type 6 SCAR products) produced DON, apart from one isolate from France, G2 which produced NIV.

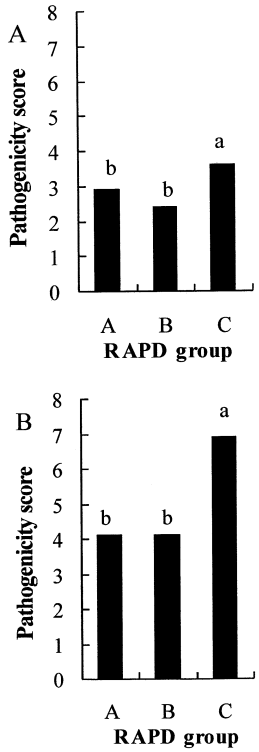

Figure 3. The mean pathogenicity scores of isolates of *F. graminearum* from the three RAPD groups, A, B and C to (A) wheat and (B) maize. A score of 0 represents no symptoms and 9 a necrotic plant. Numbers above the columns indicate significant differences at a 5% level.

Discussion

The species-specific SCAR polymorphic marker allowed the identification of 118 isolates to be confirmed and their genetic variability at one locus to be assessed. All 53 isolates from the USA and Europe, except for D2 from Germany, had a similar sized SCAR product. Isolates from Nepal appeared to be more variable at this locus than the isolates from the USA and Europe with five alleles being detected. On the basis of this assessment and the geographical origin of the isolates, the RAPD profiles of 42 selected *F. graminearum* isolates indicated that at least three groups, termed A, B and C, each supported by bootstrap analysis, could be identified. Group A could possibly be subdivided further into two subgroups, as the branch length between the two isolates RL1 and RL2, which grouped together with 100% bootstrap support, and the rest of the group was twice that of any other branch length within the group. In addition RL1, RL2 and RL3, which had previously been shown to have similar RAPD profiles and had been isolated from the same site (Carter et al., 2000), yielded the only type 1 SCAR products found outside group C. Further isolates from Nepal yielding type 1 SCAR products will be required to resolve this grouping. RAPD groups A and B were congruent with those described for Nepal previously (Carter et al., 2000) and were differentiated from group C, which contained all of the isolates from Europe and the USA. RAPD group C may be congruent with the lineage 7 defined by O'Donnell et al. (2000) who described a single population of *F. graminearum* present across Northern Europe and the USA based on the DNA sequence analysis of six genes from three loci.

The present report and that of O'Donnell et al. (2000) are consistent with the RAPD analysis of *F. graminearum* populations from Canada (Dusabenyagasani et al., 1999). The relationship between RAPD groups A and B and the proposed biogeographical lineages of O'Donnell et al. (2000)

Table 4. Analysis of variance for the pathogenicity of chemotypes of *F. graminearum* within RAPD group A towards wheat and maize

	Wheat				Maize			
	d.f.[a]	m.s.[b]	v.r.[c]	*F*. pr.[d]	d.f.[a]	m.s.[b]	v.r.[c]	*F*. pr.[d]
Chemotype	2	1.3950	1.86	0.195	2	31.057	8.23	0.005
Residual	13	0.7494			13	3.774		
Total	15	0.8355			15	7.412		

[a]Degrees of freedom; [b]mean square; [c] *F* ratio; [d] *P* value from *F* distribution.

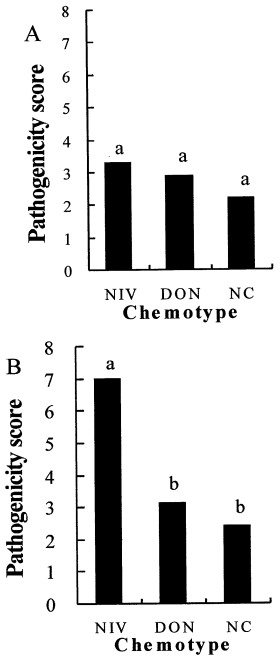

Figure 4. The mean pathogenicity scores of isolates of *F. graminearum* with the three chemotypes NIV, DON and NC from RAPD group A to (A) wheat and (B) maize. A score of 0 represents no symptoms and 9 a necrotic plant. Numbers above the columns indicate significant differences at a 5% level.

remains unclear as although both studies placed isolates from Nepal into two distinct groups, it is not clear if these groups are congruent. Two Nepalese isolates, MK6 (ARS 28723) and ML1 (ARS 28720) were used both in O'Donnell et al. (2000) and Carter et al. (2000) and were placed in RAPD group B, lineage 2 and RAPD group A, lineage 6 respectively, suggesting congruity between these groups and lineages. However a third isolate, ML13 (ARS 28721), placed in group B on the basis of its RAPD profile in this study, a classification consistent with both its SCAR product and its IGS RFLP profile (Carter et al., 2000) was described as a hybrid between lineages 2 and 6 (O'Donnell et al., 2000). Further work is required to clarify the relationship between groups determined by the RAPD assay and the proposed biogeographical lineages (O'Donnell et al., 2000). Interestingly lineage 2 (O'Donnell et al., 2000) was the only lineage reported as containing no DON producers, a similarity shared by RAPD group B. A type 2 SCAR product, only previously detected for group B isolates, has been obtained for one non-Nepalese *F. graminearum* isolate from maize in Honduras (Nicholson et al., 1998). Although RAPD data are not available for this isolate, the uniqueness of this PCR product to RAPD group B indicates that this RAPD group represents a population with a wider geographical distribution than has been detected in this study. It is, however, clear that a northwest European/northern American grouping exists as only one of several groups from around the world.

The three *F. graminearum* groups identified by RAPD analysis did not share the same pathogenicity to wheat and maize seedlings. Isolates from within populations have been shown to vary in pathogenicity to rye (Miedaner et al., 2000; Schilling et al., 1997) but differences between populations have not previously been described. *F. graminearum* group C was significantly more pathogenic to both wheat and maize than either groups A or B, although the pathogenicity of individual isolates within each group also varied.

There was also a relationship between the genetic and chemotypic variation detected within the *F. graminearum* isolates used in our study. All the toxigenic isolates in RAPD group B were NIV producers and of the 21 isolates in RAPD group C, 20 were DON producers, with the remaining isolate, G2, a NIV producer. Previous studies of *F. graminearum* chemotypes have shown that in the USA, Europe and South America the DON chemotype is more frequently detected than the NIV chemotype (Lori et al., 1992; Miedaner et al., 2000). The opposite is true in Japan, with the NIV chemotype being more prevalent (Ichinoe et al., 1983).

Within *F. graminearum*, it has been suggested that NIV and DON-producing chemotypes show no host specialisation towards maize, wheat, triticale or rye (Logrieco et al., 1990) and that the level of toxin production may be as, or more important than, the type of toxin produced (Atanassov et al., 1994). In the current study, when DON, NIV and non-classified isolates from group A were compared, isolates with a NIV chemotype were found to be more pathogenic to maize, but there was no relationship between chemotype and pathogenicity to wheat. No quantitative measurements of toxin production were made and it is unclear if chemotype, the level of toxin production, or a genetically linked trait are responsible for the higher level of pathogenicity of these isolates towards maize. Isolates within group A had originally been isolated from maize, rice and wheat and isolates were equally likely to have been obtained from any of these three hosts (Carter et al., 2000). The host of origin was not significantly related to the pathogenicity of isolates in the seedling assays.

F. graminearum isolates may be divided into groups on the basis of their RAPD profiles. Isolates from the USA and North-West Europe formed a single group, group C, while isolates from Nepal were more varied and formed two distinct groups, A and B. There were biologically significant differences between the groups, with group C isolates being more aggressive towards both wheat and maize than groups A and B. It may be hypothesised that group C represents a recently evolved, more highly pathogenic lineage, whose spread may account for the increased incidence of *F. graminearum.* This difference in pathogenicity between *F. graminearum* groups confirms the need for the vigilant monitoring of potentially infected material and selection of suitable plant breeding strategies. In addition to variation in pathogenicity, group C and group B were largely composed of isolates of a single chemotype, DON and NIV, respectively. Further work is required to study the relationship between toxigenicity and host preference. Similarly, the role of sexual recombination within and between these populations requires further study for a fuller understanding of this economically important pathogen.

Acknowledgements

This work was supported by the Department for Environment, Food and Rural Affairs, UK and the USDA, USA. J.P. Carter was supported by EU grant ERBC18-CT98-0312. The collection of fungi in Nepal by A.E. Desjardins was supported by the United States Fulbright Foreign Scholarship Programme and by the Nepalese Agricultural Research Council. We would like to thank Stephanie Folmer for technical support.

References

Aoki T and O'Donnell K (1999) Morphological and molecular characterisation of *Fusarium pseudograminearum* sp. nov., formally recognized as the Group 1 population of *F. graminearum*. Mycologia 91: 597–609

Atanassov Z, Nakamura C, Mori N, Kaneda C, Kato H, Jin Y-Z, Yoshizawa T and Mural K (1994) Mycotoxin production and pathogenicity of *Fusarium* species and wheat resistance to Fusarium head blight. Canadian Journal of Botany 72: 161–167

Bai G-H and Shaner G (1996) Variation in *Fusarium graminearum* and cultivar resistance to wheat scab. Plant Disease 80: 975–979

Carter JP, Razanoor HN, Desjardins AE and Nicholson P (2000) Variation in *Fusarium graminearum* isolates from Nepal associated with their host of origin. Plant Pathology 49: 1–10

Casale WL and Hart LP (1988) Inhibition of ^{3}H-leucine incorporation by trichothecene mycotoxins in maize and wheat tissue. Phytopathology 78: 1673–1677

Cundliffe E, Cannon M and Davis J (1974) Mechanism of inhibition of eukaryotic protein synthesis by trichothecene fungal toxins. Procedings of the National Acadamy of Science, USA 71: 30–34

Desjardins AE, Proctor RH, Bai G-H, McCormick SP, Shaner G, Buechley G and Hohn TM (1996) Reduced virulence of trichothecene non-producing mutants of *Gibberella zeae* in wheat field tests. Molecular Plant–Microbe Interactions 9: 775–781

Dusabenyagasani M, Dostaler D and Hamelin RC (1999) Genetic diversity among *Fusarium graminearum* strains from Ontario and Quebec. Canadian Journal of Plant Pathology 21: 308–314

Ehrlich KC and Daigle KW (1985) Protein-synthesis by mammalian-cells treated with C-3-modified analogs of the 12, 13-epoxytrichothecenes T-2 and T-2 tetraol. Applied and Environmental Microbiology 50: 914–918

Francis RG and Burgess LW (1975) Surveys of *Fusaria* and other fungi associated with stalk rot of maize in eastern Australia. Australian Journal of Agricultural Research 26: 801–807

Harris LJ, Desjardins AE, Plattner RD, Nicholson P, Butler G, Young JC, Weston G, Proctor RH and Hohn TM (1999) Possible role of trichothecene mycotoxins in virulence of *Fusarium graminearum* on maize. Plant Disease 83: 954–960

Ichinoe M, Kurata H, Sgiura Y and Ueno Y (1983) Chemotaxonomy of *Gibberella zeae* with special reference to production of trichothecenes and zearalenone. Applied and Environmental Microbiology 46: 1364–1369

Joffe AZ (1986) *Fusarium* Species: Their Biology and Toxicology, New York, John Wiley & Sons

Logrieco A, Manka M, Altomare C and Bottalico A (1990) Pathogenicity of *Fusarium graminearum* chemotypes towards corn, wheat, triticale and rye. Journal of Phytopathology 130: 197–204

Lori GA, Carranza MR, Violante A, Rizzo I and Alippi H E (1992) *Fusarium* spp. en trigo, capaciad toxicogenica y quimiotaxonomia de las cepas aisladas en la Argentina. Agronomie 12: 459–467

Marasas WFO, Nelson PE and Toussoun TA (1984) Toxigenic *Fusarium* Species: Identity and Mycotoxicology, The Pennsylvania State University Press, University Park, PA, USA

Miedaner T, Reinbrecht C and Schilling AG (2000) Association among aggressiveness, fungal colonization, and mycotoxin production of 26 isolates of *Fusarium graminearum* in winter rye head blight. Journal of Plant Disease and Protection 107: 124–134

Miedaner T and Schilling AG (1996) Genetic variation of aggressiveness in individual field populations of *Fusarium graminearum* and *Fusarium culmorum* tested on young plants of winter rye. European Journal of Plant Pathology 102: 823–830

Miller JD, Greenhalgh R, Wang YZ and Lu M (1991) Trichothecene chemotypes of three *Fusarium* species. Mycologia 83: 1221–130

Nicholson P, Lees AK, Maurin N, Parry DW and Rezanoor HN (1996) Development of a PCR assay to identify and quantify *Microdochium nivale* var. *nivale* and *Microdochium nivale* var.

majus in wheat. Physiological and Molecular Plant Pathology 48: 257–271

Nicholson P, Simpson DR, Weston G, Rezanoor HN, Lees AK, Parry DW and Joyce D (1998) Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Physiological and Molecular Plant Pathology 53: 17–37

O'Donnell K, Kistler HC, Tacke BK and Casper HH (2000) Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proceedings of the National Acadamy of Science USA 97: 7905–7910

Ou SH (1985) Rice Diseases. Commonwealth Mycological Institute, Kew, England

Parry DW, Jenkinson P and McLeod L (1995) *Fusarium* ear blight (scab) in small grain cereals – a review. Plant Pathology 44: 207–238

Perkowski J, Kiecana I, Schumacher U, Muller H-M, Chelkowski J and Golinski P (1997) Head infection and accumulation of Fusarium toxins in kernels of 12 barley genotypes inoculated with *Fusarium graminearum* isolates of two chemotypes. European Journal of Plant Pathology 103: 85–90

Proctor RH, Hohn TM and McCormick SP (1995) Reduced virulence of *Giberella zeae* caused by disruption of a trichothecene toxin biosynthetic gene. Molecular Plant–Microbe Interactions 8: 593–601

Schilling AG, Miedaner T and Geiger HH (1997) Molecular variation and genetic structure in field populations of *Fusarium* species causing head blight in wheat. Cereal Research Communications 25: 549–554

Schilling AG, Moller EM and Geiger HH (1994) RAPDs of *Fusarium culmorum* and *F. graminearum*. Application for genotyping and species identification. In: Schots A, Dewey FM and Oliver RP (eds) Modern Assays for Plant Pathogenic Fungi (pp 47–56) CAB International, Oxford, UK

Shifrin VI and Anderson P (1999) Trichothecene mycotoxins trigger a ribotoxic stress response that activates c-Jun N-terminal kinase and p38 mitogen-activated protein kinase and induces apoptosis. Journal of Biological Chemistry 274: 13985–13992

Simpson DR, Rezanoor HN, Parry DW and Nicholson P (2000) Evidence for differential host preference in *Microdochium nivale* var. *majus* and *Microdochium nivale* var. *nivale*. Plant Pathology 49: 261–268

Vigier B, Reid LM, Seifert KA, Stewart DW and Hamilton RI (1997) Distribution and prediction of *Fusarium* species associated with maize ear rot in Ontario. Canadian Journal of Plant Pathology 19: 60–65

Walker SL, Leath S, Hagler WM and Murphy JP (2001) Variation among isolates of *Fusarium graminearum* associated with fusarium head blight in North Carolina. Plant Disease 85: 404–410