Project Report

Regional variation among *Fusarium* spp. causing dry rot of potato

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Acknowledgements

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1 Executive summary

Over the 3-year survey 10,950 tubers (comprising 219 samples) were collected and processed to recover dry rot-producing pathogens. In total, 217 *Fusarium* isolates were recovered (i.e. the recovery rate for *Fusarium*, per tuber, was approximately 2%). *Fusarium coeruleum* was the most commonly isolated species in each survey year. On average, *Fusarium culmorum* and *avenaceum* were the next most common species, but their prevalence varied greatly by season. *Fusarium sulphureum* was the least common species on average. However, the proportion of *F. sulphureum* recovered compared with the other species remained remarkably consistent, comprising around 13% of the *Fusarium* species isolated.

Selected isolates were evaluated for their ability to produce rots *in planta* using potato tubers. *Fusarium sulphureum* was a more aggressive pathogen (producing larger rots) than the other *Fusarium* species tested. In conventional pathogenicity tests, *F. avenaceum* and *F. culmorum*, were relatively weaker pathogens. However, these species were aggressive on some cultivars, notably Hermes. An attempt has been made in this report to quantify the importance of different species in the *Fusarium*-dry rot complex for the main potato cultivars tested.

The selected isolates were also assessed for their sensitivity to the fungicides, thiabendazole and imazalil. Particular emphasis was placed on evaluating the potential resistance to imazalil among isolates of *F. avenaceum* using *in vitro* tests. Experiments were conducted to ascertain the effect of storage temperature (4, 7 and 10°C) on the development of dry rot caused by different *Fusarium* species. Generally, the extent of rot development increased with increasing storage temperature.

The Scottish Crop Research Institute (SCRI), in collaboration with Higgins GI [Alves, IV30 8UP, UK], undertook a research project (SAPPIO LINK 81) which developed molecular based diagnostic methods to detect a range of fungal pathogens causing rots, including *Fusarium* spp. These molecular techniques were used in the work presented in this report to confirm the morphologically based identification of *Fusarium* species. Molecular diagnostic assays were also used to detect *Fusarium* species directly from tuber skin. The relative frequencies of *Fusarium* species found using these assays were comparable with those found by the isolation method (except in the case of *F. culmorum*). However, the direct molecular detection method was around 25 times more sensitive than the isolation method.

The main findings of the study were:

- The relative prevalence of Fusarium species:
    - Fusarium coeruleum was the most commonly isolated species in each survey year, comprising 58, 51 and 39% of the Fusarium species isolated from the 2000, 2001 and 2002 crops respectively.
    - On average, *Fusarium culmorum* and *avenaceum* were the next most common species (comprising, respectively, 20 and 16% of the total species between 2000 and 2002), but their prevalence varied markedly from year to year.
    - *Fusarium sulphureum* was the least common species on average. Moreover, this species was not detected on Scottish crops.
- All isolates of *Fusarium sulphureum* tested were insensitive to thiabendazole. All isolates of *F.avenaceum, F. coeruleum* and *F. culmorum* were fully sensitive to thiabendazole.

- All isolates of the four *Fusarium* species tested were sensitive to imazalil. However, *F. avenaceum* isolates had a broad range of activity (as measured by their IC_{50}) to imazalil suggesting some isolates might be best described as 'moderately' sensitive to the fungicide.

- The susceptibility to dry rot varied considerably between cultivar. Also, this difference was not consistent between *Fusarium* species. Indicating that it is not possible to assume that resistance to one species confers resistance to all *Fusarium* species. The cultivar, Hermes, was susceptible to all *Fusarium* species at a typical processing storage temperature.

Based on this work, the recommendations include:

- Diagnosing which species of *Fusarium* is/are present on tuber surfaces would help determine risk of dry rot developing in susceptible cultivars.

- In cases where *F. avenaceum* is present, a mixture of imazalil and thiabendazole is recommended for control. Thiabendazole is unlikely to prevent infection caused by *F. sulphureum*.

- Be aware of the risk of dry rot developing even if storing at temperatures below 10°C. As always, the advice is to ensure skins have adequately set, keep damage to a minimum and, if necessary, dry cure crop into store.

2 Introduction

Dry rot, caused by *Fusarium* spp., is one of the most important fungal storage rots affecting potato. Recent reports show that in SE England 70% of ware crops and 100% of seed stocks were affected by the disease, and that the disease itself was present on as many as 1% of tubers (Bradshaw, Turner & Elcock, 2001). As the presence of rots on tubers automatically excludes them from sale, this represents potential losses of up to 35,000 tonnes of stored potatoes annually. In addition, dry rot on seed tubers can cause blanking although the economic importance of this is difficult to quantify.

Dry rot in the UK is generally attributed to fungus *F. coeruleum*. However, since the early 1980s there have been no comprehensive assessments of the species that cause dry rot. The intervening period has been one of rapid change in the potato industry with the widespread use of cold storage, changes in fungicide usage and the introduction of new potato cultivars with unknown levels of resistance to dry rot.

Gibberella and *Nectria* are sexual, or teleomorph, states of *Fusarium* species. The nomenclature of the *Fusarium*-group is complex and taxonomists normally use the teleomorph names (i.e. *Gibberella* or *Nectria*) to characterise a species. However, most plant pathologists know individual species under their *Fusarium* anamorph (or asexual) name. Throughout this report, the anamorph names will be used. This raises one point of confusion, namely that the name *F. sulphureum* has been used for species belonging to the *Gibberella pulcaris* teleomorph. However, other authors use the anamorph name, *F. sambucinum* to describe the asexual form of *G. pulcaris* pathogens causing dry rot on potatoes. For the purposes of this report, it should be assumed that *F. sambucinum* and *F. sulphureum* are synonymous. The following sexual states for *Fusarium* species, commonly associated with potato dry rot are: *F. avenaceum* = *G. avenacea*; *F. coeruleum* = unknown (likely to be *Nectria haematococca*); *F. culmorum* = unknown (likely to be *G. zeae*); and *F. sulphureum* = *G. pulcaris*.

Fusarium coeruleum has predominated the dry rot-causing *Fusarium* species in the UK since the 1970s (Brenchley & Wilcox, 1979). Carnegie *et al*. (SASA, unpublished) found that 53% of the Scottish Elite stocks in 1992-93 were contaminated by *F. coeruleum*. However, the incidence of other rot-causing

Fusarium spp. was not noted. Despite isolated outbreaks of disease caused by the highly pathogenic species *F. sulphureum*, workers (Hide *et al*., 1992; Carnegie, SASA, pers. comm) have also noted a high incidence of *F. avenaceum* in Scotland associated with post-storage rotting.

Cultivars vary in their resistance to different *Fusarium* spp. (Corsini and Pavek, 1986), and cultivars brought into a particular region may be exposed to a *Fusarium* spp. uncommon in their region of origin, and to which they may have little resistance. Different *Fusarium* spp. also vary in their amenability to chemical control (Hide and Cayley, 1980; Hide *et al*, 1992). For example, Hide *et al.* (1992) found that 68% of *F. sulphureum* isolates were resistant to thiabendazole whilst *F. coeruleum* isolates were fully sensitive. However, where isolates were recovered from thiabendazole-treated stores, the level of thiabendazole resistance in *F. sulphureum* increased to 100%. Thus using an inappropriate fungicide may result in sub-optimal disease control, which is linked with the development of fungicide resistance.

As well as causing unsightly rots, themselves sufficient to cause rejection, all four of the main dry rot-causing *Fusarium* spp. that affect potato are capable of producing mycotoxins (Blaney, 1991). These toxic metabolites are damaging to human and animal health. However, it should be noted that whilst there have been no reported cases of mycotoxin contamination of GB potatoes, there have been incidences of mycotoxin poisoning in other countries. *Fusarium sulphureum* produces trichothecenes, a toxin group of which diacetoxyscirpenol (DAS) is the major toxin (Jelen *et al*, 1995). *Fusarium coeruleum* also produces scirpenols. *Fusarium culmorum* strains that are able to produce trichothenes have also been found on wheat (Jennings *et al*, 2004). As yet, no trichothene-producing strains of *F. avenaceum* have been found. However, *F. avenaceum* produces the toxins, fusarin and moniliformin (Desjardins, 2003) amongst others.

The objectives of the study presented here are:

- To quantify the Fusarium spp. causing dry rot and determine the extent of regional variation within the Fusarium dry rot complex.

- To identify specific control strategies for dry rot based on information gained on the interactions between Fusarium spp., potato cultivar, fungicides and crop storage.

3 Materials and methods

3.1 Survey and sample collection

3.1.1 Sample collection

Tuber samples were collected in 2001, 2002 and 2003 from four main GB potato-growing regions (Scotland, east; England, east; England, south east; and England, south west). Each sample consisted of five, ten-tuber sub-samples of asymptomatic material. Varieties chosen to be collected were the commonly grown processing cultivars (Estima, Maris Piper, Saturna). However, other varieties were also collected as required. Participating agronomists and store managers from a number of leading processors and suppliers collected samples and provided information on supplied survey questionnaire, provenance, fungicide and storage treatments on survey questionnaire forms (Appendix 1).

3.1.2 Storage and maintenance of samples

Samples were stored at 3.5°C (no humidity control) prior to wounding. The samples were loaded into store from March to April and stored for up to 10 weeks before wounding. Stores were controlled using a conventional temperature control and monitoring system [Cornerstone Systems Ltd] operating in refrigeration mode. Control was done on the basis of crop and ambient temperatures. Stores had probes buried in the crop to monitor and record store temperatures.

3.2 Fusarium isolation and identification

Tubers (unwashed, but excess soil removed using clean paper towel) were wounded to a depth of 4mm using a flame-sterilised wounding device (consisting of 4, 3mm diameter pins forming corners of a square with 20mm sides). Two sets of four wounds were applied per tuber (placed on opposite sides). After the incubation period (minimum 6 weeks at 10^{o}C), tubers were cut through the wounding sites using a clean, flame-sterilised cabbage knife. Tissue from the leading edge of tissue showing dry rot symptoms(four pieces per rot) were plated out onto $^{1}/_{4}$ strength potato dextrose agar plus streptomycin ($^{1}/_{4}$ PDA) (Oxoid, Basingstoke, UK) and incubated at 16°C. The cultures were then grown on SNA, a minimum nutrient medium, and potato dextrose agar (PDA) and identified using characteristics of colony morphology, conidia and conidiogenous cells. Single spore colonies were produced for each *Fusarium* isolate. A representative number of isolates that had been identified on the basis of their cultural and morphological characteristics were tested using a PCR-based diagnostic assay (carried out at SCRI).

3.2.1 Fusarium identification using PCR diagnostics

Fungal cultures were grown on PDA (Difco Labs, UK). Mycelial growth was removed using a sterile scalpel and resuspended in a 2ml microcentrifuge tube containing 0.5 ml CTAB extraction buffer (2 % CTAB, 1.5 M NaCl, 0.2 M Tris, 25 mM EDTA; pH 8.0) and 0.1 g of 1.0 mm glass beads. Cell lysis was performed using a Mini-BeadBeater-1 at 5000 rpm/1 min or at a Medium setting/1 min using Mini-BeadBeater-8. Tubes were centrifuged at 13k/4 min to pellet cell debris and the supernatant was transferred to a new 2 ml tube. One volume (0.5 ml) of chloroform was added to each supernatant and vortex mixed before centrifugation at 13k/4 min. The aqueous phase (~0.5 ml) was removed and transferred to new 1.5 ml tube, followed by a 1/10 volume (50 µl) of 3 M NaAc (pH 5.4) and one volume (550 µl) of Isopropanol. Samples were incubated at room temperature for a minimum of 1 h, before tubes were centrifuged at 13k/4 min, supernatant removed, and the pellets washed in 100 µl 70 % ethanol. Tubes were re-centrifuged (13k/1 min) and the ethanol was removed. DNA pellets were resuspended in 50 µl TE buffer and stored at -80 ^{o}C until required.

The standard PCR amplification of all DNA extract samples was based on an initial denaturation at 95 ^{o}C (2 min), followed by 37 cycles of denaturation at 95 ^{o}C for 45s, annealing at 60 ^{o}C for 1 min (or 65 ^{o}C for *F. culmorum*), extension at 72 ^{o}C for 90 s, and a final elongation at 72 ^{o}C for 5 min in a reaction volume of 25 µl using a GeneAmp PCR System 9600 thermal cycler (Applied Biosystems). Optimal conditions for PCR (single-round and nested) contained a master mix of the following components: 1 x reaction buffer (16 mM $[NH_4]_2SO_4$, 67 mM Tris-HCl pH 8.8, 0.1 % Tween-20; Bioline UK Ltd), 200 µM each dNTPs (Bioline), 0.3 µM of each primer (MWG-BIOTECH UK Ltd), 5.0 mM $MgCl_2$, 250 µg ml^{-1} BSA (Boehringer Mannheim, UK), 1U Biotaq Diamond (Bioline). One µl of undiluted or 1/10 diluted DNA (representing 10-100 ng) was used as template and 1 µl of single-round PCR product was used for nested PCR when using the Universal *Fusarium* primers. DNA of each appropriate *Fusarium* spp. was used as positive control in the PCR assay; negative controls were carried out with PCR reagents and 1 µl dH_2O or non-target DNA. PCR products were analysed by electrophoresis on a 2 % agarose gel in 1 X TBE buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA, pH 8.0), stained with ethidium bromide (0.5 mg l^{-1}), and photographed under UV illumination.

TABLE 1. CONVENTIONAL PCR PRIMERS DESIGNED FOR THE SPECIFIC DETECTION OF *FUSARIUM* SPECIES CAUSING DRY ROT OF POTATOES.

Pathogen	Primers	Product size (bp)
Fusarium coeruleum	Fcoer1F1 Fcoer2R1	271
Fusarium sulphureum	Fsulp1F1 Fsulp2R1	291
Fusarium avenaceum (Turner *et al,* 1998)	JIAf JIAr	294
Fusarium culmorum (Nicholson *et al,* 1996)	Fc01F Fc01R	592
'Universal *Fusarium'* Primers	Funi1F1 Funi2R1	~ 470

3.3 Pathogenicity testing

3.3.1 A comparison of varietal susceptibility to Fusarium spp.

Ten varieties were tested (Hermes, Russet Burbank, Estima, Desiree, Sante, Lady Rosetta, Maris Piper, Cara, Marfona, and Saturna). Tubers were hand washed, surface sterilised with 70% IMS then wounded to a depth of 4mm using a 4 mm diameter steel pin. A 20µl drop of conidial suspension (approx. 5.0 x 10^4 conidia/ml) was pipetted onto each wound. Tubers were left to dry for ½ hour. Uninoculated but wounded tubers were used as controls. Wounded tubers were placed in clean, labelled, paper bags and incubated at 10°C. After 6 weeks incubation, each tuber was cut across the wound and the width and depth of rotted tissue was measured. Rots were assumed to be conical for the purposes of analysis. Therefore, the volume of each rot was calculated using the equation:

$$\textbf{Volume} = \pi r^2 (h/3)$$

Where ***r*** is ½ the width of rot and ***h*** is the depth of rot.

3.3.2 Effects of temperature on development of dry rot

Tubers were wounded (as described in Section 3.3.1) and inoculated with suspensions of *Fusarium coeruleum*, *F. avenaceum* and *F. sulphureum* at 5.0 x 10^5 conidia per ml; and *F. culmorum* at 2.0 x 10^5 conidia per ml as described in Section 3.3.1. Uninoculated tubers were also used as controls. Wounded tubers were placed in clean, labelled, paper bags and incubated in 3-tonne experimental stores at 4, 7 and 10°C. After 6 weeks incubation, each tuber was cut across the wound and the width and depth of rotted tissue was measured. The volume of rot was calculated using the equation in Section 3.3.1.

3.4 Fungicide sensitivity testing

The sensitivity of the isolates to TBZ and imazalil was assessed by taking plugs (6 mm diameter) from 10 day old colonies and placing them on Petri dishes containing approximately 10 ml of malt extract agar (MEA) to which 0, 0.3, 3 or 30 mg of TBZ; or 0, 0.1, 1.0 or 10 mg of imazalil per litre MEA had been added. Two replicate plates of each fungicide concentration were prepared for each isolate. The diameter, across two perpendicular axes, of developing colonies on inoculated MEA plates was assessed after 5 days at 16°C. From these measurements, the concentration of fungicide required to inhibit the growth of each isolate by 50% (IC_{50}) was calculated.

3.5 Statistical analysis

Statistical analyses were carried out using Genstat (release 6.1, Lawes Agricultural Trust [Rothamsted Research]). A stepwise analysis of deviance (using a Generalised Linear Model) was used to compare data for *Fusarium* recovery between years, regions, seed source, storage temperature and seed. Initially, maximal models were produced (Appendix 2), from these, optimal models were derived by removing factors that did not contribute significantly to the models.

4 Results

4.1 Survey

Over the 3-year survey 10,950 tubers (comprising 219 samples) were collected and processed to recover dry rot-producing pathogens. In total, 217 *Fusarium* isolates were recovered (i.e. the recovery rate for *Fusarium*, per tuber, was approximately 2%). *Fusarium coeruleum* was the most commonly isolated species in each survey year, comprising 58, 51 and 39% of the *Fusarium* species isolated from the 2000, 2001 and 2002 crops respectively (Table 2). On average, *Fusarium culmorum* and *avenaceum* were the next most common species (comprising, respectively, 20 and 16% of the total species between 2000 and 2002), but their prevalence varied markedly from year to year. *Fusarium sulphureum* was the least common species on average. However, the proportion of *F. sulphureum* recovered compared with the other species remained remarkably consistent (comprising 15, 12 and 14% of the *Fusarium* species isolated from the 2000, 2001 and 2002 crops respectively).

The direct PCR test on potato tubers was in broad agreement with the traditional isolation method when testing the 2001 crop (Table 2). For example, *F. coeruleum* was the most prevalent species using both methods. Also, *F. sulphureum* and *avenaceum* were found in approximately the same proportions using both tests. However, there was a disagreement between the traditional and PCR methods regarding the recovery of *F. culmorum*. No *F. culmorum* was detected by PCR in DNA from tuber skins. . There were initial problems with the PCR protocols for that species (i.e. *F. culmorum* requires larger reaction volumes and annealing temperatures) but these problems were resolved and amplification of the DNA extracts on two further occasions did not detect *F.culmorum* despite amplification in the positive control samples. The differences could be caused by non-diagnosis using PCR; levels of *F.culmorum* occurring on the tuber surface could be below the level detectable by the assay, or the differences could be real as the tubers sampled were different, although from the same stocks. A sub-sample of eight isolates that had been identified by SBEU as *F. culmorum* was sent to SCRI for diagnosis. All eight isolates were confirmed as *F. culmorum* by PCR.

TABLE 2: PROPORTION (EXPRESSED AS %) OF *FUSARIUM* SPP. BY SEASON.

***Fusarium* species**	**2000-01 (isolations)**	**2001-02 (isolations)**	**2001-02 (PCR)**	**2002-03 (isolations)**	**3-year mean/totals***
F. coeruleum	58	51	*83*	37	49
F. culmorum	9	37	*0* †	12	19
F. sulphureum	15	13	*13*	13	14
F. avenaceum	16	0	*4*	31	16
Fusarium other	3	0	-	7	3
Total tubers tested (Total isolates)	4100 (69)	2100 (63)	*50 (-)*	4750 (82)	10950 (217)

† Protocols specific to *F. culmorum* were not optimised at time of testing. * mean/totals of isolations

4.1.1 Regional variation in Fusarium species

Ignoring differences between years, the incidence of *F. avenaceum, sulphureum* and *culmorum* differed between regions (P<0.001) (Table 3). However, the incidence of *F. coeruleum* did not differ between regions (P=0.616).

TABLE 3: MEAN INCIDENCE (%) OF *FUSARIUM* SPECIES BY REGION (MEANS OF 2000 TO 2002 CROPS). VALUES IN BRACKETS ARE STANDARD ERRORS (P=0.05).

Pathogen	England, east	England, south east	England, west & south west	Scottish, east
F. avenaceum	0.34 (0.181)	0.28 (0.154)	2.31 (2.055)	0.17 (0.130)
F. coeruleum	1.11 (0.232)	0.87 (0.194)	1.04 (0.288)	0.81 (0.149)
F. culmorum	0.21 (0.125)	0.84 (0.225)	0.67 (0.333)	0.06 (0.044)
F. sulphureum	0.38 (0.369)	1.97 (1.886)	0.28 (0.262)	<0.01 (0.002)

Fusarium coeruleum was ubiquitous in 2000, 2001 and 2002 crop samples collected throughout the four GB regions (Figs 1 to 3). Moreover, the pathogen was the most common species of *Fusarium* detected on crops grown in Scotland. *Fusarium sulphureum* was absent from 2000, 2001 and 2002 Scottish crops. However, the pathogen was found in all English regions (except the south west 2001 crop). *Fusarium avenaceum* was not detected on 2001 crops. The incidence of *Fusarium avenaceum* and *culmorum* were extremely variable between region and year (P<0.001).

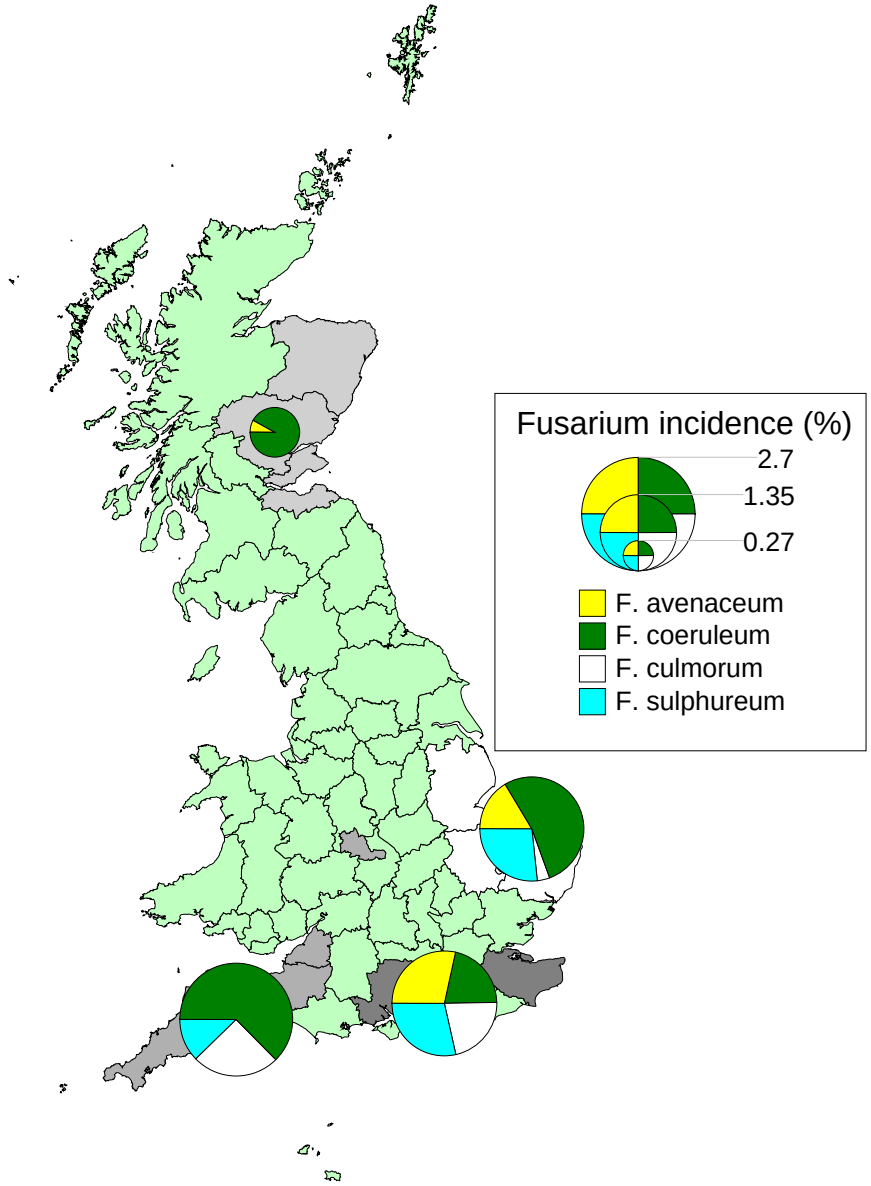

FIGURE 1. INCIDENCE OF FUSARIUM SPECIES AS INOCULUM ON TUBERS (2000 CROP) COLLECTED FROM FOUR GB POTATO-GROWING REGIONS.

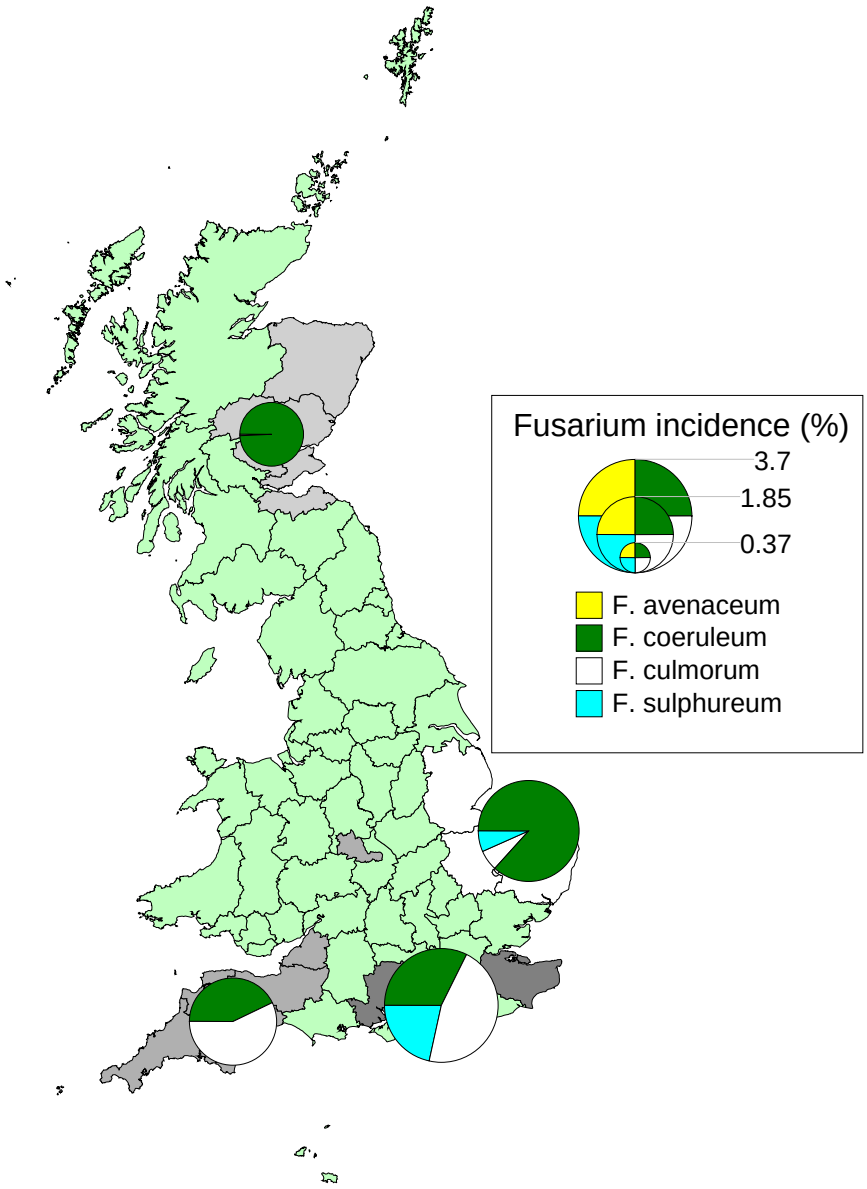

FIGURE 2. INCIDENCE OF FUSARIUM SPECIES AS INOCULUM ON TUBERS (2001 CROP) COLLECTED FROM FOUR GB POTATO-GROWING REGIONS.

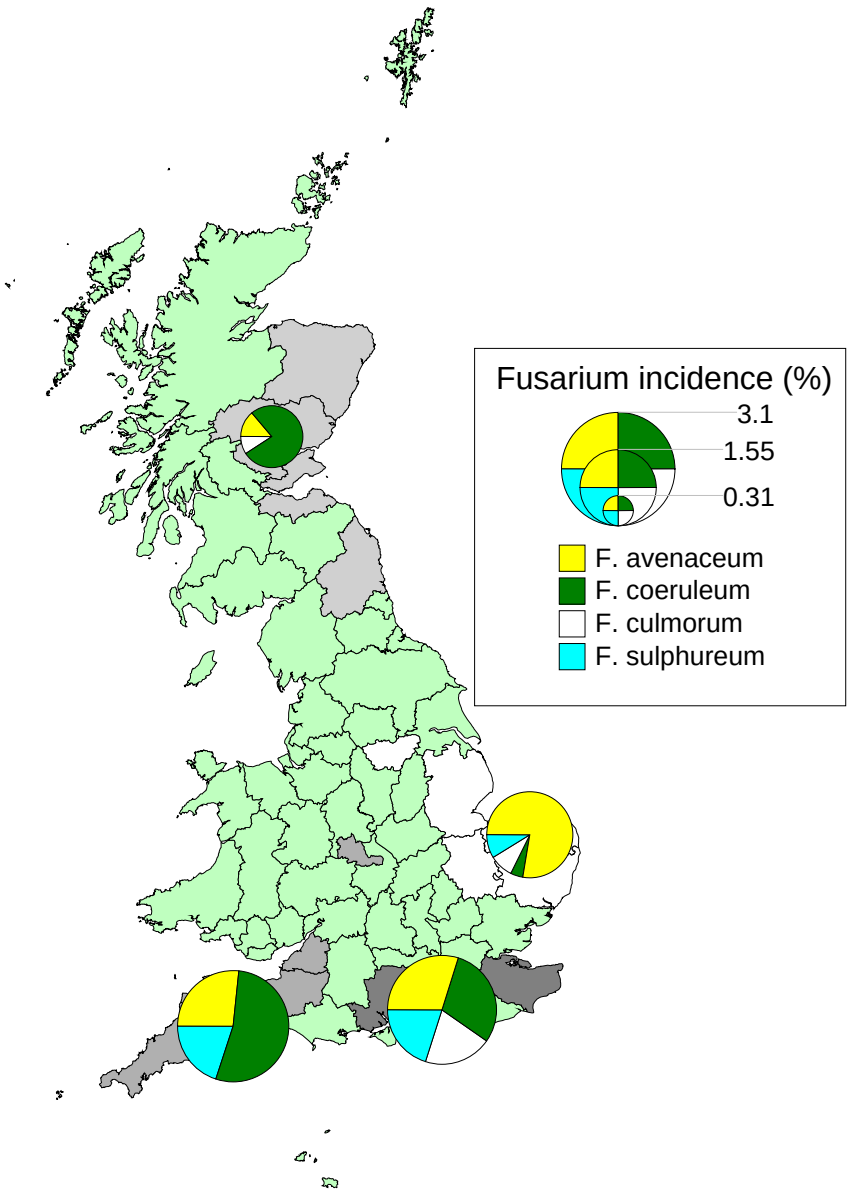

FIGURE 3. INCIDENCE OF FUSARIUM SPECIES AS INOCULUM ON TUBERS (2002 CROP) COLLECTED FROM FOUR GB POTATO-GROWING REGIONS.

4.1.2 Correlations between Fusarium species and agronomic and storage factors

4.1.2.1 Fusarium sulphureum

The incidence of *F. sulphureum* varied by region, seed source and seed fungicide. Regional variation accounted for the largest effect on the incidence of sulphureum ($P<0.001$), where the highest levels of pathogen were found on tubers grown in the south east and the least on tubers grown in Scotland. Where the seed had been grown also affected recovery ($P=0.002$). Dutch seed had higher levels of *F. sulphureum* than GB-grown seed. The type of seed fungicide also affected recovery ($P=0.001$), where TBZ applied to seed increased the level of *F. sulphureum* compared with imazalil and no fungicide treatments. Cultivar, storage temperature and crop type (seed or ware/processing) did not affect the recovery of *F. sulphureum*.

4.1.2.2 Fusarium coeruleum

The incidence of *F. coeruleum* was affected by cultivar ($P<0.001$), but only because one variety, Wilja, had higher levels of *F. coeruleum* than the other cultivars. The incidence of *F. coeruleum* varied slightly from year to year ($P=0.011$) but was not influenced by region ($P=0.616$), seed source ($P=0.362$), seed fungicide ($P=0.253$), storage temperature ($P=0.117$) and crop type ($P=0.333$).

4.1.2.3 Fusarium avenaceum

Year, region, seed fungicide and seed source affected the level of *F. avenaceum*. Year had the largest effect on levels ($P<0.001$). Region also had a large effect on levels ($P<0.001$), where crops grown in the west and south west had higher levels than elsewhere. Also, English-grown seed had higher levels of *F. avenaceum* than other sources ($P<0.001$). Treating seed with imazalil increased the level of *F. avenaceum* ($P=0.002$) compared with nil fungicide or TBZ (Fig. 5).

4.1.2.4 Fusarium culmorum

The level of *F. culmorum* varied by year ($P<0.001$) and region ($P<0.001$). There were higher levels of pathogen on tubers grown in the England, west & south west, and south east regions compared with Scottish east and England, east regions.

4.2 Fusarium *isolation and identification – comparison of traditional methods with PCR-based diagnostic methods*

PCR diagnostic tests were done directly on a sub-sample of the 2001 crop and the results were compared with that of the traditional isolation method. The PCR method detected *Fusarium* species in 78% of the tubers, whereas the isolation plate method recovered *Fusarium* from 1.6% of tubers (Table 1; Appendix 1). The PCR test detected *F. avenaceum* at low incidence levels (4% of tubers) where no *avenaceum* had been detected by the isolation plate method. However, the DNA-based diagnostic test did not detect *F. culmorum*, which had been isolated by the traditional method.

4.3 Pathogenicity

4.3.1 Pathogenicity of four Fusarium species at 4, 7 and 10°C

The degree of rot development in cv Maris Piper tubers depended on the species of *Fusarium* that was present near wounds ($P<0.001$). The volume of rot (cm^3) was greatest in tubers that had been inoculated with *F. sulphureum* conidia, compared with other *Fusarium* species tested at 4, 7 and 10°C (Fig 4). *Fusarium coeruleum* was the next most aggressive species. The *Fusarium* species, *avenaceum* and *culmorum*, produced very little rotting at the conidial concentrations used (1.0 to 5.0 x 10^5 conidia/ml). Storage temperature also had a large influence on rot development ($P<0.001$), where the volume of rot increased with increasing temperature. However, there was an interaction between species and

temperature, where the aggressiveness of *F. avenaceum* and *F. culmorum* was poor at all temperatures tested.

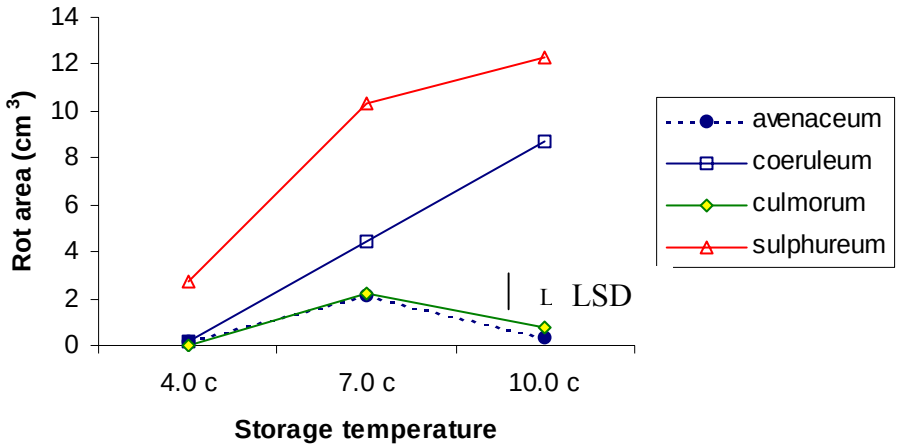

FIGURE 4. VOLUME OF ROT PRODUCED IN CV MARIS PIPER TUBERS BY FOUR SPECIES OF *FUSARIUM* AT 4, 7 AND 10°C. VALUES ARE THE MEAN OF 4 ISOLATES.

4.3.2 Sensitivity of commonly grown cultivars to four Fusarium species

The volume of rot varied considerably between cultivar. This difference was not consistent between Fusarium species. Indicating that it is not possible to assume that resistance to one species confers resistance to all Fusarium species. The experiment was not designed to directly compare the aggressiveness of the four Fusarium species tested because conidial concentrations were not equivalent. However, F. sulphureum was the most aggressive species in all ten cultivars.

TABLE 4. AN ASSESSMENT OF CULTIVAR SUSCEPTIBILITY TO FOUR SPECIES OF *FUSARIUM*. ROT VOLUME (CM^3) INDUCED BY INOCULATING TUBERS WITH *FUSARIUM* CONIDIA. DATA ARE THE MEANS ACROSS FOUR ISOLATES FOR EACH SPECIES.

	***Fusarium* species**			
Cultivar†	***F. avenaceum***	***F. coeruleum***	***F. culmorum***	***F. sulphureum***
Burbank	0.1	5.0	3.2	67.8
Hermes	15.6	2.4	11.9	29.2
Marfona	2.9	13.9	0.0	37.9
Estima	16.4	5.5	6.1	26.3
Desiree	11.0	6.8	0.1	12.6
Cara	4.5	7.7	0.0	16.0
Piper	3.1	13.1	0.1	11.6
Rosetta	4.3	0.1	2.0	17.3
Sante	11.2	2.8	0.0	4.2
Saturna	1.3	3.9	1.2	7.1
$LSD_{p=0.05;\ 120\ df}$	5.4	3.3	11.5	16.5

† The cultivar order is ranked by total dry rot volume produced by the four *Fusarium* species.

4.4 Fungicide sensitivity

All isolates of *Fusarium sulphureum* tested were insensitive to thiabendazole (Table 5). All isolates of *F. avenaceum, F. coeruleum* and *F. culmorum* were fully sensitive to thiabendazole.

All isolates of the four *Fusarium* species tested were sensitive to imazalil. However, *F. avenaceum* isolates segregated into two groups: those that had a relatively high response to imazalil (i.e. IC_{50} values of <5.0 mg/l) and those that had a relatively low response (i.e. IC_{50} values of >6.0 mg/l).

TABLE 5. SUMMARY RESULTS FOR THIABENDAZOLE SENSITIVITY TESTING

Species	Number tested	IC_{50} range (mg/l)	% sensitive
F. avenaceum	10	2.0 – 2.5	100
F. coeruleum	11	1.5 – 2.0	100
F. culmorum	12	1.5 – 2.0	100
F. sulphureum	13	>20	0
Total	46		-

TABLE 6. SUMMARY RESULTS FOR IMAZALIL SENSITIVITY TESTING

Species	Number tested	IC_{50} range (mg/l)	% sensitive
F. avenaceum	10	1.4 – 6.3	100 (?)
F. coeruleum	11	2.4 – 3.5	100
F. culmorum	12	0.3 – 0.8	100
F. sulphureum	13	1.3 – 1.8	100
Total	46		-

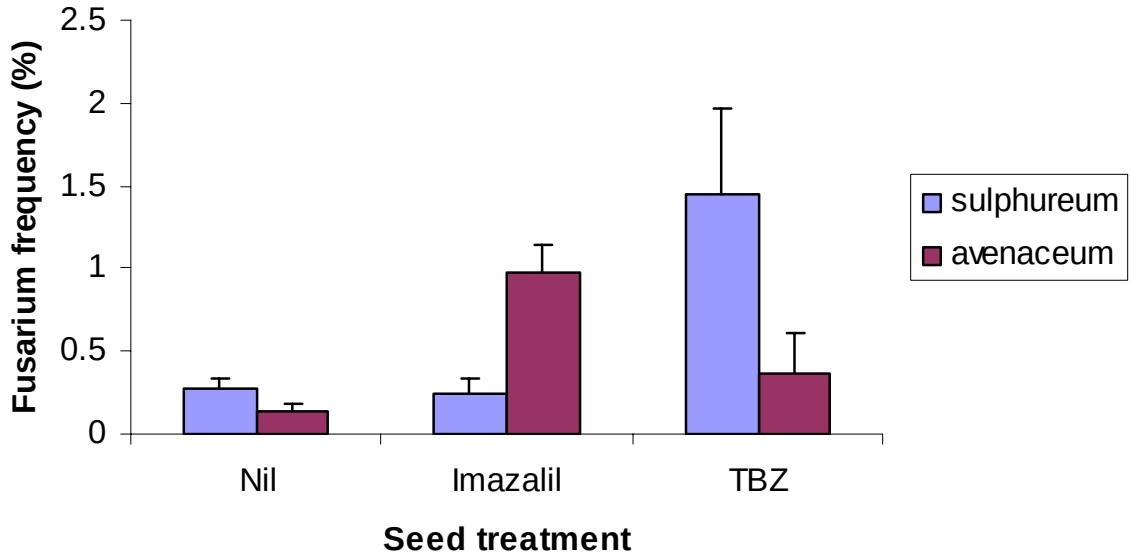

FIGURE 5. EFFECT OF FUNGICIDE TREATMENT ON SEED ON FREQUENCY OF *FUSARIUM AVENACEUM* AND *F. SULPHUREUM* ON DAUGHTER TUBERS FROM SUBSEQUENT CROP. T-BARS REPRESENT THE STANDARD ERRORS OF THE MEAN (*P*=0.05)

5 Discussion, conclusions & recommendations

5.1 Discussion

Fusarium coeruleum was the most commonly isolated *Fusarium* species in the material collected from the 2000 to 2002 crops (representing approximately 49% of the *Fusarium* population). This is consistent with previous findings (Hide *et al*, 1992; Brenchley & Wilcox, 1979). The species, *F. avenaceum* and *F. culmorum*, varied considerably from year to year but over three years, these species represented approximately 18%, each, of the *Fusarium* population. The isolation rate of *F. sulphureum* remained constant from one year to the next. This was generally the least common species isolated (at around 13%). The relative frequency of species isolations obtained from the 2001 samples using the traditional isolation method (i.e. the method used in this survey) were compared with that using a direct PCR test on potato tubers. The results were broadly comparable. The PCR diagnostic test failed to detect *F. culmorum* but, otherwise, was 25 times more sensitive than the traditional isolation method. However, an unpublished survey funded through the SAPPIO LINK programme, carried out on tubers with rots during 2000/2001, found that *F. avenaceum* was the most common species and *F. sulphureum* the least common. The results from both this BPC-funded survey and the SAPPIO LINK survey show the large variation in the presence of each *Fusarium* species from one stock to the next and highlights the low level of *F. sulphureum* relative to the other species.

The source of seed was found to be important in determining what species of *Fusarium* predominates. For example, daughter tubers from Dutch seed had higher levels of *F. sulphureum* than from GB-grown seed. However, care needs to be taken in interpreting the data. The amount of Dutch seed tested was low compared with GB-grown seed; only four samples of Dutch-sourced seed was found to have *F. sulphureum* compared with 26 samples of GB-sourced seed. Also, where seed had been grown was confounded with fungicide use: the majority of Dutch seed had been treated with thiabendazole. *Fusarium sulphureum* was found to be insensitive to thiabendazole both in the work presented here and in previous studies (Hide *et al*, 1992). This fits with the results presented in this report that thiabendazole use was correlated with increased frequencies of *F. sulphureum*. Therefore, it is impossible to determine whether increases in *F. sulphureum* associated with Dutch seed was due to seed location or seed fungicide treatment.

When cv Maris Piper was inoculated with suspensions of *F. sulphureum* or *F. coeruleum*, rot development was greater at 10°C than 4 or 7°C. *Fusarium sulphureum* was consistently more pathogenic on cv Maris Piper than *F. coeruleum* at all three temperatures tested. The species, *F. avenaceum* and *F. culmorum*, produced little, if any, rots on cv Maris Piper. In addition, *F. sulphureum* was more pathogenic than the other three species on eight out of ten cultivars at 10°C.

All 13 isolates of *Fusarium sulphureum* tested were insensitive to thiabendazole. A similar number of isolates of *Fusarium avenaceum*, *coeruleum* and *culmorum* were fully sensitive to thiabendazole. All isolates of the four *Fusarium* species tested were sensitive to imazalil. However, *F. avenaceum* isolates segregated into fully sensitive and partially sensitive groupings. Interestingly, the survey data suggests that a higher proportion of tubers had *F. avenaceum* when seed stocks were treated with imazalil than in those that were not treated with imazalil. This might indicate that insensitivity to the fungicide is building up within *F. avenaceum*. However, the assumption is based on a regression-type analysis of the data. It is impossible to predict whether the correlation between imazalil treatment and presence of *F. avenaceum* is direct or indirect. For example, it is possible that seed suppliers who apply imazalil are those who already have a dry rot problem but it is unlikely that this would be restricted to those affected by *F. avenaceum*.

Fusarium sulphureum was identified as a particularly important species because of its aggressiveness and insensitivity to thiabendazole. Interestingly, *F. sulphureum* was not found on any of the Scottish samples tested during the three-year survey. However, the direct PCR diagnostic test, which had been carried in parallel by SCRI, did detect *F. sulphureum* on one Scottish stock. Therefore, the data presented in this survey indicates that *F. sulphureum* may be present on Scottish stocks but at lower levels than the other main *Fusarium* species.

5.2 Conclusions

Fusarium coeruleum was the most commonly isolated species in each survey year. *Fusarium sulphureum* was the least common species.

All isolates of *Fusarium sulphureum* tested were insensitive to thiabendazole. All isolates of *F.avenaceum, F. coeruleum* and *F. culmorum* were fully sensitive to thiabendazole.

All isolates of the four *Fusarium* species tested were sensitive to imazalil. However, *F. avenaceum* isolates had a broad range of inhibitory effect in the presence of imazalil suggesting some isolates might be best described as 'moderately' sensitive to the fungicide.

The susceptibility to each *Fusarium* species varied considerably between cultivar. Also, this difference was not consistent between *Fusarium* species. Indicating that it is not possible to assume that resistance to one species confers resistance to all *Fusarium* species. The cultivar, Hermes, was susceptible to all *Fusarium* species at a typical processing storage temperature.

Fusarium sulphureum was not detected on Scottish crops by the direct isolation method. However, a direct PCR diagnostic test suggests this species is present (possible at low frequencies) in Scottish stocks.

5.3 General recommendations

When growing susceptible varieties, identifying and quantifying the *Fusarium* species present on the tuber surfaces would help determine the risk of dry rot developing.

Thiabendazole or imazalil as single seed treatments increases the probability of detecting *F. sulphureum* and *F. avenaceum* respectively on tubers. It is suggested that a mixture of thiabendazole and imazalil should be considered in **high risk** situations. A Specific Off-Label Approval (SOLA) has been granted in the UK (Anon., 2004) to allow the continued use of thiabendazole and imazalil mixtures for the control of dry rot in seed potatoes. This follows the loss of registration of *Extratect* [Syngenta Crop Protection Ltd, Whittlesford, CB2 4QT, UK] the only proprietary formulation available containing both active ingredients, which is no longer being manufactured.

As always, for early lifted crops, ensure skins are set, damage is kept to a minimum, and dry cure into store if possible.

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7 Appendix 1

Store sampling form

Study 210 **DRY ROT STORE SAMPLING FORM** **Season**

GROWER: **REGION:**

FIELD NAME (& OS grid ref. if available):

VARIETY: **WARE/SEED**

STOCK REFERENCE: **CERTIFICATE No. (if known):**

SEED SOURCE: Mixed ☐ Scottish ☐ English ☐ Dutch ☐ Home ☐

SEED GRADE:

HANDLING: Graded ☐ Whole Crop ☐

STORAGE: Refrigerated ☐ Ambient ☐ Holding temp: ^{o}C

FUNGICIDES: Storite ☐ (TBZ) Fungazil ☐ (Imazalil) Extratect ☐ (TBZ/imazalil) Rovral ☐ (Iprodione) Other ☐ None ☐

SAMPLE NUMBER: 210/1_ _ _ **DATE COLLECTED**:

COLLECTOR: **SUPPLIER NAME**:

PLAN OF STORE/LOCATION OF SAMPLES:

7 Appendix 2

Generalised Linear Model – accumulated analysis of deviance

The deviance tables below show the strength of effect of each factor on the frequency of each *Fusarium* species. The tables can be interpreted in the same way as an analysis of variance except that with an analysis of deviance, the distributions have approximate χ^2 distributions (Payne *et al*, 1994). Note, the tables shown represent the *maximal* models. However, probability levels presented in the Section 4 were calculated by stripping away non-significant factors, to leave the *optimal* model for each species.

I) *Fusarium sulphureum*

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Year	2	1.8694	0.9347	0.93	0.393
+ Variety	12	22.6393	1.8866	1.89	0.031
+ Region	3	30.8796	10.2932	10.29	<.001
+ seed_source	6	29.4710	4.9118	4.91	<.001
+ fungicide_on_seed	5	12.7910	2.5582	2.56	0.025
+ temp_category	2	2.4581	1.2290	1.23	0.293
+ seed_ware	2	0.3819	0.1909	0.19	0.826
Residual	186	68.4432	0.3680		
Total	218	168.9336	0.7749		

II) *Fusarium coeruleum*

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Year	2	9.075	4.537	4.54	0.011
+ Variety	12	54.840	4.570	4.57	<.001
+ Region	3	1.793	0.598	0.60	0.616
+ seed_source	6	6.578	1.096	1.10	0.362
+ fungicide_on_seed	5	6.593	1.319	1.32	0.253
+ temp_category	2	4.290	2.145	2.15	0.117
+ seed_ware	2	2.201	1.100	1.10	0.333
Residual	186	269.299	1.448		
Total	218	354.669	1.627		

III) *Fusarium culmorum*

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Year	2	28.3851	14.1925	14.19	<.001
+ Variety	12	15.0177	1.2515	1.25	0.240
+ Region	3	25.5606	8.5202	8.52	<.001
+ seed_source	6	17.7894	2.9649	2.96	0.007
+ fungicide_on_seed	5	6.9773	1.3955	1.40	0.222
+ temp_category	2	2.8663	1.4332	1.43	0.239
+ seed_ware	2	1.6531	0.8266	0.83	0.438
Residual	186	81.2229	0.4367		
Total	218	179.4724	0.8233		

IV) *Fusarium avenaceum*

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Year	2	28.2080	14.1040	14.10	<.001
+ Variety	12	21.6081	1.8007	1.80	0.042
+ Region	3	42.0453	14.0151	14.02	<.001
+ seed_source	6	15.1120	2.5187	2.52	0.019
+ fungicide_on_seed	5	13.9359	2.7872	2.79	0.016
+ temp_category	2	3.0243	1.5121	1.51	0.220
+ seed_ware	2	4.8202	2.4101	2.41	0.090
Residual	186	110.6702	0.5950		
Total	218	239.4240	1.0983		

