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SURVEY OF *FUSARIUM* SPECIES INFECTING WINTER WHEAT IN ENGLAND, WALES AND SCOTLAND, 1989 & 1990

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Final report of a two year project conducted by several collaborating organisations throughout the United Kingdom. The work commenced in February, 1989 and was supported by a total grant of £256,458 (£215,821 to MAFF, Central Science Laboratory; £36,640 to The Scottish Agricultural College - Edinburgh; £3,997 to Cranfield Institute of Technology) from the Home-Grown Cereals Authority (Project No. 0005/1/89).

Abstract

*Fusarium**species infecting winter wheat were surveyed in fields from the Agricultural Development and Advisory Service (ADAS) Regions and Scotland at growth stages (GS) 31 and 73 in 1989 and 1990 and in grain samples taken from the same fields at harvest. Assessments of stem-base diseases at GS 31 were based on definitions and photographs of 9 symptoms of disease in 1989 and 10 in 1990. *F. nivale* was the most prevalent of the *Fusarium* species isolated from the stem base at both GS 31 and 73. *F. culmorum* and *F. avenaceum* were also apparent on the stem base, the former increasing in incidence at GS 31 in 1990. These two species increased in prevalence at the later growth stage. *F. poae* was the most prevalent species on the ears. *In vitro* sensitivity tests using a range of fungicides on isolates obtained at GS 31 showed high levels of sensitivity to prochloraz and varying levels of sensitivity of *F. nivale* to benomyl.

F. culmorum was the predominant species in grain samples from England and Wales in 1989 and as prevalent as *F. poae* in 1990. *F. nivale* was also relatively common in grain samples particularly in Scotland. However, overall levels of both *Fusarium* and blackpoint (*Alternaria alternata*) in grain were generally low. One grain sample from Scotland contained a *Fusarium* toxin and a second contained a toxin derived from *Alternaria*. Trichothecenes were detected in 25% of the wheat grain samples from England and Wales and zearalenone in one. Of the *Fusarium* species tested isolates of *F. poae* produced the most toxic of the mycotoxins.

The possible influence of environmental factors on the incidence and severity of *Fusarium* species, the level of mycotoxin production, and the implications of control measures on stem base disease interactions and crop establishment are discussed.

*In 1988 the International Commission on the Taxonomy of Fungi renamed *Fusarium nivale*, *Microdochium nivale* (perfect stage = *Monographella nivalis*).

F. avenaceum

Symptoms at all stages are similar to those caused by *F. culmorum*. Howeyer *F. avenaceum* is less able to survive in soil, and is generally less virulent than *F. culmorum*.

F. graminearum

Seedlings: Forms short lived chlamydospores and is seed-borne. May cause severe root rot or seedling disease resulting in pre- or post-emergence death, but mainly under warm, wet conditions. Uncommon in the UK.

Young plants (GS 30-32): Symptoms rare (unreported).

Anthesis to milk development (GS 61-79): Can cause a foot rot leading to the sudden death of mature plants. Uncommon in the UK.

Ears and grain: Causes an ear blight ("Scab") in which fungal growth may be evident. Under warm wet conditions, dark purple perithecia of *Gibberella zeae* form on the infected ears and stem bases. Affected grains may be shrivelled with a red discolouration. Mycotoxins are often found.

F. poae

Seed-borne, but symptoms only apparent on the ears, where it causes lesions with a bleached centre and dark brown margin on the glumes. *F. poae* is commonly isolated from grain and is an important source of mycotoxins.

It is clear from these descriptions that *Fusarium* diseases can occur at all stages of crop development, and different species are implicated in causing damage at each stage. In the UK, the most important species which cause disease are *F. nivale*, *F. culmorum*, *F. graminearum*, *F. avenaceum* and *F. poae*. Other saprophytic Fusaria are also frequently encountered on the stem base. The relative importance of *Fusarium* species and the damage which they cause has not been fully investigated.

*In 1988 the International Commission on the Taxonomy of Fungi renamed *Fusarium nivale*, *Microdochium nivale* (perfect stage = *Monographella nivalis*).

2. SURVEY OF *FUSARIUM* SPECIES INFECTING WINTER WHEAT AT GROWTH STAGE 31

2.1 Objective

To describe disease symptoms and to determine the range and incidence of *Fusarium* species infecting winter wheat at GS31.

2.2 Introduction

There is little published data on the incidence of *Fusarium* spp. on winter wheat during the early stages of crop growth. In surveys undertaken at the seedling stage in Scotland from 1971 to 1974, *F. nivale* was found to be the most commonly occurring *Fusarium* species being isolated from 80-90% of the crops. *F. culmorum* or *F. avenaceum* were isolated from 25-45% of crops (Rennie *et al.*, 1983). In an intensive study of the incidence of *Fusarium* species in winter wheat, Parry (1990) sampled 3 crops at monthly intervals from January to August over a three year period (1987-1989). He found that *F. nivale* was the predominant species during the spring in 1988 and 1989. However in 1987, *F. avenaceum* was more frequent than *F. nivale* in May, although *F. nivale* subsequently became predominant.

Visual identification of the causes of cereal foot rots, which may often be a complex of several diseases, is frequently problematic and inaccurate. During early growth stages symptoms of eyespot, sharp eyespot (caused by *Pseudocercosporella herpotrichoides* and *Rhizoctonia cerealis* respectively) and *Fusarium* spp. are often indistinct and easily confused. Detailed descriptions of the symptoms associated with cereal foot rots particularly those caused by *Fusarium* species are therefore needed in order that further investigations can be carried out into the epidemiology of *Fusarium* stem base diseases. It is currently recommended that, for the purposes of making decisions on fungicide applications, eyespot assessments are undertaken during stem extension (GS30-37) (Goulds & Polley, 1990). GS31 is thus a significant stage in crop growth when fungicide spray programmes are likely to begin and was therefore selected for investigation to determine the incidence of *Fusarium* symptoms and the species present.

2.3 Materials and Methods.

The survey of early season stem-base diseases of winter wheat was carried out in April when crops were at the first node stage (GS31). Farm addresses derived from lists used in the annual ADAS winter wheat disease surveys (Polley & Thomas, 1991) were sent to ADAS Regional Plant Pathologists responsible for the nine regional areas. Samples from Scotland were taken by staff at the Scottish Agricultural College (Edinburgh and Aberdeen). The regional areas and the survey sample distribution in 1989 and 1990 were as follows:

1990	1989	Symptom definition
1	1	**Eyespot alone.** Honey -brown discoloured area which may have developed into a distinct, often eye-shaped lesion. There may be a "pupil" of dark grey to black fungal growth which cannot be easily rubbed off.
2	2	**Eyespot + *Fusarium*.** Honey-brown lesions, but with dark grey or black discolouration around the margins.
3	-	***Fusarium*.** Date brown discolouration of whole or part of the leaf sheath at the lowest internode (basal browning).
4	3	***Fusarium*.** Charcoal grey discolouration of the leaf sheath at the lowest internode.
5	4	***Fusarium*.** Charcoal grey discolouration at the junction of the leaf blade and leaf sheath.
6	5	***Fusarium*.** Date-brown discolouration at the margins of the leaf sheath.
7	6	**Vascular discolouration.** Indistinct brown vascular discolouration on the leaf sheath.
8	7	**Sharp eyespot alone.** Lesions with bleached, sometimes shredded centres and thin, well-defined reddish-brown margins.
9	8	**Sharp eyespot + *Fusarium*.** Distinct sharp eyespot lesions, but with dark grey to black discolouration around the margins.
10	9	**Small discrete lesions.** These have a pale brown to grey centre and a well defined dark-brown border.
11	-	**Varietal pigmentation.**
12	10	**Symptomless.**

Isolations were made from lesions found on each sample up to a maximum of 15 thought to be *Fusarium*, 5 eyespot and 5 sharp eyespot. In addition, isolations were made from 5 symptomless tillers from each sample to determine whether any latent infection was present. In 1989, if the number of symptomless tillers in the random sample was less than 5, then additional symptomless tillers were selected to increase the number to a total of 5. In 1990 additional symptomless tillers were not selected but instead additional *Fusarium* symptoms were isolated if the total was less than 15.

All tissues were surface sterilised in a 10% sodium hypochlorite solution (1-1.4% available chlorine) for 3 minutes and divided into four pieces before plating out. Isolations were carried out on sucrose nutrient agar (SNA) (Nirenberg, 1981) by placing four pieces of tissue on each plate. The plates were incubated at 17^{o} C under near ultra-violet light (12 hours light/12 hours dark cycle) for 14 days. Isolates of *Pseudocercosporella* spp. and *Rhizoctonia* spp. were identified by microscopical examination of the agar plate from the underside.

(b)1990

The percentage number of tillers recorded in each symptom category in 1990 is shown in Table 4. 92.5% of crops examined showed symptoms of *Fusarium* infection. Eyespot was recorded on 15.8%, sharp eyespot on 10.0% and *Fusarium* symptoms on 23.6% of the 3,850 tillers examined. 77 isolates of *Fusarium* were obtained from 532 lesions and symptomless tissues, an isolation rate of 14.5%. Seven different species of *Fusarium* were isolated of which *F. nivale* was the most prevalent representing 49.4% of all *Fusarium* species isolated (Table 5). This corresponded to 7.1% of all isolations. *F. culmorum* was recovered from 4.7% of lesions.

Table 6 shows the % recovery of *Pseudocercosporella* spp., *Rhizoctonia* spp. and *Fusarium* species from each symptom type. Results show that the highest level of *Rhizoctonia* spp. (56.3%) was isolated from symptom 8 (sharp eyespot). *Pseudocercosprella herpotrichoides var acuformis* was isolated most frequently from symptom 1 (44.6% of lesions) and symptom 2 (41.6%) and also from symptom 9 (sharp eyespot + *Fusarium*) suggesting some confusion between eyespot and sharp eyespot symptoms. *Pseudocercosporella herpotrichoides var herpotrichoides* was also isolated from tissues with symptom types 1 and 2 but at a much lower frequency. *Pseudocercosporella herpotrichoides var anguioides* was not isolated in 1990. The Fusarium symptoms 2-6 and 9 were found to be associated with the highest frequencies of *Fusarium* . Symptoms associated with charcoal grey discolouration of the leaf sheath and basal browning (4, 5 and 6) yielded the highest numbers of *Fusarium* isolates. Vascular discolouration (symptom 7) was also associated with a relatively high frequency of *F. nivale* and *F. culmorum*. *F. culmorum* was also recovered from sharp eyespot + *Fusarium* lesions (symptom 9). Saprophytic Fusaria were most commonly recovered from sharp eyespot lesions.

2.5 Discussion

These data show that at growth stage 31, *F. nivale* was the predominant *Fusarium* species accounting for 46.9% of the *Fusarium* isolates recovered in 1989 and 49.4% in 1990. This species was particularly associated with charcoal grey lesions or date-coloured lesions on the margins of the leaf sheaths and was isolated from 2.1% of all stem-base lesions in 1989 and from 7.1% in 1990. *Fusarium culmorum* and *F. avenaceum* were more prevalent in 1990 than in 1989. The predominance of *F. nivale* in the *Fusarium* population may indicate that this species is an early coloniser and may also be more tolerant of the cooler conditions in early spring than the other species. Isolation of *F. culmorum* was significantly higher in 1990 compared to 1989 possibly due to two consecutive years of mild winters and high summer temperatures. It has been reported that *F. avenaceum* is a weak pathogen (Duben & Fehrmann, 1979) and it may be significant that this species was rarely isolated and then mainly in conjunction with sharp eyespot (1989) or eyespot symptoms (1990). Saprophytic

Table 1 % of stems affected by stem base disease classified in symptom categories 1 - 10 (1989)

Symptom category	% stems affected
1 Eyespot	5.9
2 Eyespot + *Fusarium*	13.4
3 *Fusarium* (leaf sheath)	10.5
4 *Fusarium* (leaf sheath/blade)	2.9
5 *Fusarium* (leaf sheath margins)	6.2
6 Vascular	14.4
7 Sharp eyespot	5.2
8 Sharp eyespot + *Fusarium*	4.4
9 Small lesions	4.2
10 Symptomless	32.8

Number of stems assessed = 7,266

Table 2 Number of isolates of *Fusarium* species recovered from lesions at GS31 as a % of the total number of *Fusarium* isolates obtained and as a % of the total number of lesions plated out (1989)

Species	Number recovered from lesions (inc. symptomless)	As % of total *Fusarium* isolates	As % of total lesions (inc. symptomless) plated out
F. nivale	122	46.9	2.1
F. culmorum	24	9.2	0.4
F. graminearum	11	4.2	0.2
F. avenaceum	5	1.9	0.09
F. poae	1	0.4	0.02

Total number of lesions (including symptomless) = 5,768

Total number of Fusaria isolated = 260

Table 4 % of stems affected by stem base disease classified in symptom categories 1 - 12 (1990)

Symptom category		% stems affected
1	Eyespot	9.0
2	Eyespot + *Fusarium*	6.8
3	*Fusarium* (basal)	10.3
4	*Fusarium* (leaf sheath)	0.3
5	*Fusarium* (leaf sheath/blade)	0.8
6	*Fusarium* (leaf margins)	2.8
7	Vascular	7.6
8	Sharp eyespot	7.4
9	Sharp eyespot + *Fusarium*	2.6
10	Small discrete lesions	0.3
11	Varietal pigmentation	2.0
12	Symptomless	50.2

Number of stems assessed = 3,850

Table 5 Number of isolates of *Fusarium* species recovered from lesions at GS31 as a % of the total number of *Fusarium* isolates obtained and as a % of the total number of lesions plated out (1990)

Species	Number recovered from lesions (inc. symptomless)	As % of total *Fusarium* isolates	As % of total lesions (inc. symptomless) plated out
F. nivale	38	49.40	7.10
F. culmorum	25	32.46	4.69
F. graminearum	0	0.00	0.00
F. avenaceum	3	3.90	0.56
F. poae	1	1.29	0.19

Total number of lesions (including symptomless) = 532

Total number of Fusaria isolated = 77

3. SURVEY OF FUSARIUM SPECIES INFECTING WINTER WHEAT AT GROWTH STAGE 73-75

3.1 Objective

To determine the range and incidence of *Fusarium* species infecting winter wheat GS73.

3.2 Introduction

ADAS winter wheat disease surveys are undertaken annually at growth stage 73-75 (Polley & Thomas 1991) when the kernels are milky-ripe. This is the stage at which grain dry matter is accumulating most rapidly and diseases are therefore expected to be having their most depressant effect on yield (King, 1980). Data from the 1989 and 1990 surveys (Figs 26 and 27) show that *Fusarium* symptoms in these two years have been at least as prevalent as eyespot at GS 73-75, but this ignores the fact that many crops are sprayed against eyespot. Figure 28 shows that there can be considerable variation in the incidence of *Fusarium* from year to year, with 1989 and 1990 having particularly high levels. In order to undertake experimental work on the epidemiology of *Fusarium* and the damage which is caused, it is first necessary to determine which *Fusarium* species are associated with stem base symptoms at this growth stage.

3.3 Materials and Methods

Each field sampled at growth stage 31 was re-sampled at GS 73-75, as part of the ADAS Winter Wheat Disease Survey. The distribution of samples is shown below:

Region	Area	ADAS Centre	Total samples 1989	Total samples 1990
N (N)	North	Newcastle	10	7
N (L)	North	Leeds	13	10
M & W	Midlands	Wolverhampton	22	27
EAST	East	Cambridge	63	51
SE (W)	S. East	Wye	9	9
SE (R)	S. East	Reading	14	10
SW (B)	S. West	Bristol	16	10
SW (SC)	S. West	Starcross	3	3
WALES	Wales	Cardiff Trawsgoed	12	6
SCOT	Scotland	SAC (Edinburgh & Aberdeen)	33	20
Total			195	153

Fourteen *Fusarium* species were isolated from the nodes in 1989, 12 from the lowest internodes, 8 from the top internodes and 7 from the ears. *F. nivale* predominated on the nodes and internodes although *F. culmorum* was not uncommon. *F. poae* was the most common species found on the ear. Isolation frequencies from the top internode were very low, the highest isolation rate being 0.7% (*F. culmorum*).

Symptom assessments in 1990 showed that 45.9% of tillers were affected with symptoms of eyespot + *Fusarium* and 9.0% by symptoms of sharp eyespot + *Fusarium*. 93% of crops surveyed in 1990 were infected by *Fusarium* species. In 1990 isolations of *F. nivale* were fewer than in 1989 and isolations of *F. avenaceum* were more common (Table 10). Unlike 1989, *F. culmorum* and *F. avenaceum* occurred at a similar frequency to *F. nivale* on the nodes and internodes but *F. poae* was again the most common *Fusarium* species isolated from the ears. Isolations of the same disease symptoms onto a second medium, PDA, produced similar levels of isolation of *Fusarium* species compared to PPA except for an increased isolation rate of *F. nivale* (Table 11).

In 1990, stems were divided into slight and moderate + severe symptom categories. Moderate or severe nodal symptoms were found on 8.1% of tillers showing symptoms. Isolations showed that *F. nivale*, *F. culmorum* and *F. avenaceum* were the species most commonly isolated from these lesions although *F. nivale* was slightly more common than the other two (Table 12). An ad hoc test on a small number of severely affected nodes from one sample produced isolates of *F. nivale* only. A total of 50.9% of moderate or severe symptoms were found to be infected by *Fusarium* spp. Moderate or severe internodal infection was found on 10.5% of affected tillers of which 28.6% were confirmed to be infected by *Fusarium* spp.. *F. nivale* predominated in the *Fusarium* population and was isolated from 11% of tillers showing moderate or severe internodal symptoms and represented 38.5% of all Fusaria isolated from this symptom category.

There were few obvious differences between regions in the frequency of the species encountered on the stems and ears of winter wheat. In both 1989 (Table 13) and 1990 (Tables 14 and 15) there was a higher level of *F. nivale* and a lower level of *F. poae* in Scotland than in the ADAS regions (England and Wales). The highest frequency of *F. culmorum* in 1989 was in the south-west but there were no obvious regional differences in the occurrence of this species in 1990. *F. avenaceum* was more prevalent in the north than elsewhere in 1989, and in Scotland, the north and midlands than in the south in 1990. In both years the highest incidence of *F. poae* occurred in the south-east.

3.4 Discussion

Less than 30% of tillers showing *Fusarium* disease symptoms were confirmed to be infected by *Fusarium* species in 1989 and 1990. However, this may be a reflection of the limitations

Table 7 National stem base and ear *Fusarium* disease incidence (1989) (average % stems or ears affected at GS73-75)

Symptom type	% stems or ears affected
Fusarium (nodal)	23.4
Fusarium (internodal)	36.4
Fusarium (ears- *F. poae* lesions)	7.2
Fusarium (ears - other)	0.7

Table 8 National stem base and ear *Fusarium* disease incidence (1990) (average % stems or ears affected at GS73-75)

Symptom type	% stems or ears affected
Fusarium (nodal)	26.2
Fusarium (internodal)	26.5
Fusarium (F. poae lesions)	10.3
Fusarium (ears - other)	0.4

Table 11 Frequency of isolation of *Fusarium* species at GS73 on PDA (1990)

	Number of isolates as % of lesions examined			
Species	Internode	Node	Top internode	Ear
F. nivale	13.0	11.6	0.0	1.2
F. culmorum	6.6	6.3	0.0	0.3
F. avenaceum	7.3	7.7	0.6	1.2
F. graminearum	0.0	0.1	0.0	0.0
F. poae	0.7	0.6	0.9	38.7
Total no. of isolations	1000	1111	326	326

Table 12 Frequency of isolation of *Fusarium* species from moderate and severe symptoms at GS73 on PDA (1990)

	Number of isolates as % of lesions examined	
Fusarium species	Nodal	Internodal
F. nivale	19.5 (38.3)	11.0 (38.46)
F. culmorum	17.0 (33.3)	8.8 (30.80)
F. avenaceum	12.7 (26.7)	5.7 (20.00)
Fusarium - others	1.7 (3.3)	3.1 (10.76)
% infection by *Fusarium* spp	50.9	28.6

Figures in parentheses = % of total *Fusarium* isolates

Table 15 Regional incidence of *Fusarium* species at GS73 on PDA (1990).

Region	Number of isolates as percentage of total no. isolations per region					
	F. nivale	*F. culmorum*	*F. aven.*	*F. gramin.*	*F. poae* (ears)	Others
North	6.7	6.4	6.0	0.0	4.87	4.1
M & W	9.3	4.4	6.0	0.0	6.24	1.2
East	10.8	4.1	5.5	0.0	4.83	1.1
S. East	6.5	5.4	0.9	0.0	9.32	1.4
S. West	7.7	6.9	3.4	0.0	6.00	2.9
Scotland	12.6	4.5	12.0	0.2	1.38	3.9

Total number of *Fusarium* isolates = 769

Total number of isolations = 2763

Total percentage recovery from symptoms = 27.83 %

years. However, in both Scotland and the ADAS regions (England and Wales) the mean percentage of grain infected overall was low with *F. nivale* predominating in Scotland and *F. poae and F. culmorum* in the ADAS regions. 33% of samples from Scotland were infected by *F. tricinctum* although the mean percentage infection was less than 1%. This appears to be the first record of this species affecting cereal crops.

4.5 Discussion

There have been few investigations in the UK on levels of *Fusarium* infection on grain. Hewett (1965) undertook a survey of the degree of *F. nivale* infection in commercial samples of seed wheat in England and Wales from 1959 to 1963. Levels of infection were low in the first four years of the survey (0.1-0.5% seed infected) but in 1963, 5.3% of grain from the cultivar Capelle Desprez was found to be infected. The suggested reason for this upsurge was prolonged snow cover in winter followed by a wet summer. The sporadic occurrence of high infection levels in other years suggested an influence of localised factors. Rennie *et al.* (1990) examined seed samples of winter wheat from Scotland harvested in 1987 and 1988 and found mean infection levels of *F. nivale* of 38% and 35% respectively. By comparison, infection in Scottish seed samples of 4% in 1989 and 8% in 1990 found in the surveys reported here are low, probably due to the relatively dry seasons prior to harvesting. This may also have been the case with samples from the ADAS regions where infection levels were even lower. Data from the Seed Testing Station in Ireland (Mangan, 1988) shows that in 1981 4% of seed from 60 crops was infected with *Fusarium*, mainly *F. nivale*. In a similar survey in 1987 34% of seed was found to be infected. Available evidence thus seems to suggest that the background level of *F. nivale* in seed may have been increasing and that a return to wetter summer conditions could see a resurgence of the disease. The loss of organomercury as an effective seed treatment could have serious consequences, particularly in the wetter parts of the country, since high levels of *F. nivale* infection can reduce the germination of winter wheat seed intended for sowing.

Parry *et al.* (1984) found that ear blight, primarily caused by *F. culmorum*, was widespread in trials in 1982. *F. avenaceum* and *F. culmorum* tend only to occur in wet seasons, and particularly when crops have lodged (Hewett, 1966). There is some indication from this survey that *F. culmorum* and *F. poae* in seed may be more prevalent in the England and Wales than in Scotland.

INFECTION OF GRAIN BY FUNGI CAUSING BLACKPOINT

4.6 Materials and Methods

89 samples of milling wheat were obtained from ADAS regions and 16 from Scotland in 1990. Four samples from one ADAS region were received in poor condition, and although

Table 16 Isolation rates of *Fusarium* species from wheat grain samples (1989)

Fusarium species	% samples infected	Range of % grain infected for infected samples	Mean % grain infected
ADAS samples (179 samples) (PDA)			
F. poae	46.67	1 - 26	3.96
F. nivale	7.78	1 - 10	3.43
F. avenaceum	1.11	1	1.00
F. culmorum	3.89	5 - 8	5.43
Scottish samples (32 samples) (PDA)			
F. poae	65.6	1 - 11	2.5
F. nivale	68.8	1 - 31	4.0
F. avenaceum	40.6	1 - 5	1.0
F. culmorum	15.6	1 - 2	<1.0

5. SURVEY OF MYCOTOXIN CONTAMINATION IN GRAIN SAMPLES AND TOXIGENIC POTENTIAL OF *FUSARIUM* AND *ALTERNARIA* ISOLATES

5.1 Objective

To determine the degree of mycotoxin contamination of grain samples and to assess the potential for mycotoxin production by *Fusarium* and *Alternaria* isolates.

5.2 Introduction

A range of mycotoxins is known to be produced by a variety of *Fusarium* and *Alternaria* species when grown in culture. Surveillance carried out in many countries has confirmed the natural occurrence of several trichothecenes, zearalone and other *Fusarium* mycotoxins in cereals and after harvest. These compounds have the potential if present in sufficient amounts to effect the health of both man and animal although the economic effects on livestock are those usually noticed.

The mycotoxins produced and their amount are influenced by the weather prevailing before and at harvest and by the fungal species present. In a number of surveys including that carried out on behalf of the HGCA in 1982, the levels found have been very low. However in others this has not been the case as in a survey carried out on Bavarian cereals from the 1987 harvest (Lepschy *et al.*, 1989) in which some samples showed very high levels of contamination including a level of 44 mg/kg of deoxynivalenol in one sample. Much less is known about the occurrence of *Alternaria* mycotoxins in cereals.

ENGLAND AND WALES (1990)

5.3 Materials and Methods

i) Receipt and storage of samples

All wheat samples received at Slough were placed immediately at -20°C until required for study. Isolates of *Fusarium* and *Alternaria* were stored at 4°C until required. Appendix 2 shows a flow diagram of the procedures used for examination of wheat samples.

ii) Examination of wheat for mycotoxins and biological activity.

a) mycotoxin extraction

Each sample was thoroughly mixed and 200g was taken and ground. A 60g sub sample was weighed and mycotoxins extracted using the solvents employed in the Patterson and Roberts (1979) method. This entails extracting with a mixture of acetonitrile and aqueous potassium chloride. The solvent extract is filtered and 100 ml taken for the determination of *Fusarium* mycotoxins and for bioassay tests. A

6x50ml portions of ethyl acetate. Solvent was removed at 35°C and the residue made up in 5 ml of methanol:acetone. This solution was used for:

i) determination of trichothecenes by GC/MS section ii)b
ii) zearalenone/-ol using HPLC section ii)c
iii) brine shrimp bioassay
iv) cytotoxicity

b) Alternaria

Each complete culture was macerated with 100 ml acetonitrile as for *Fusarium* cultures. The solution was filtered and the residue washed with 25 ml of 4% aqueous potassium chloride and 100 ml dichloromethane. After shaking, the lower organic layer was filtered through anhydrous sodium sulphate. The residue was washed with two further 50 ml portions of dichloromethane and the combined extract was evaporated to near dryness at 35°C. The residue was made up to 5 ml with dichloromethane. Aliquots of this were treated as follows: 2 ml, 1 ml and 1 ml portions were added to 3 separate 4ml capacity amber glass vials and the solution evaporated to dryness. The residue in the first vial was made up in 2 ml acetonitrile:water, 84:16 for HPLC, that in the second vial redissolved in 200 ul of ethyl acetate for cytotoxicity testing and that in the third vial in 400 ul of chloroform for brine shrimp assay. The last 1 ml aliquot remaining from the original extract was used for TLC.

c) determination of cytotoxicity

Microtitre test plates were prepared by adding 200 ul of a 10^5/ml Hep2 cell suspension to each well, followed by incubation at 37° C for 24 hours in humidified air at 5% carbon dioxide concentration. 1 ul of each of the undiluted extracts of either *Fusarium* or *Alternaria* cultures and negative controls were then added to the wells in triplicate. Controls were either ethyl acetate (*Alternaria*) or ethanol(*Fusarium*) and 2% malt medium extract. The medium was poured off the plates and drained on tissue for 1-2 minutes. 200 ul of ethanol were added to each well for 10 minutes and the plates drained and dried over cool air for 20 minutes. Giemsa stain was prepared, 20 minutes prior to use, as a 1/10 dilution in distilled water. 200 ul were added to each well for 20 minutes and the plates drained and left to dry.

Toxicity was measured as a percentage of cells adhering to the wells (cell survival) after staining. A scale of 0-4 was used, with 0 being 100% cell survival and 4 being 0% cell suvival. In the majority of the samples examined, cells survived or were all killed.

toxic to Hep2 cells. No other extract was significantly cytotoxic.The extracts from samples 332, 388, 396, 112 and 106 caused greater than 50% mortality in brine shrimp larvae tests. Sample 106 killed 98% of shrimp larvae. This was the only sample in which diacetoxyscirpenol (25 µg/kg)was identified but at a level which would not account for the toxicity found. Samples 106 and 112 both came from the same location.

iii) Mycotoxin analysis (fungal isolates)

a) *Fusarium*

Ten *F. poae*, six *F. nivale* and four *F. culmorum* isolates were examined for trichothecenes, zearalenone and zearalenol. No mycotoxins were produced by F. nivale. Two out of four *F. culmorum* produced trace levels of fusarenon-X at 2 and 3 ug/culture. Six *F. poae* cultures were completely free from mycotoxins, three had trace amounts of diacetoxyscirpenol and one (175/7) contained 117 ug of diacetoxyscirpenol.

b) *Alternaria*

HPLC results showing the amount of each *Alternaria* mycotoxin occurring in fungal cultures are given in Table 20. Quantitative results could not be obtained for altertoxin I due to a co-eluting HPLC peak and the TLC results are given for this compound and for tenuazonic acid for which an HPLC method had not been developed.

iv) Bioassay results (fungal isolates)

a) *Fusarium*

All isolates showed some toxicity to brine shrimp. The extract from the culture medium control was non-toxic. Undiluted extracts from *F. culmorum* isolates gave mortalities ranging from 32% to 99% but after dilution by a factor of ten , non were then toxic. Results for *F. nivale* were similar ranging from 33% to 100% but again non-toxic on dilution. F.poae isolates were somewhat more toxic in the range 66% to 100% with sample 175/7 giving 34% mortality after ten fold dilution.

All *Fusarium* isolates were cytotoxic but when the medium control was tested this was also found to be toxic. This is now known to be due to interference by compounds leaching from the solid phase clean-up columns used in preparation of the samples tested.

Alternaria

Examination of the toxicity of *Alternaria* culture isolates to brine shrimps showed cultures 18 and 14 to give greater than 50% mortality while numbers 20, 15 and 3 gave between 38% and 50% kills. The isolates of the stock *A. alternata* strain tested was non-toxic.

why trichothecene production was so low in these cultures; however most were cytotoxic and killed brine shrimp larvae.

No *Alternaria* mycotoxins were found in any sample of wheat. However limits of detection for the methods developed were not as sensitive as for the trichothecenes, being 25 µg/kg for altenuene and iso-atenuene, 200 µg/kg for alternariol monomethyl ether, 400 µg/kg for alternariol and 500µg/kg for altertoxins I and II. Bioassays were not required on wheat samples collected for Alternaria only.

Seven out of the 20 *Alternaria* cultures failed to grow. However,from those that did most produced several toxins and the results are shown in Table 20. Isolate 14 stands out as different from the rest both in the range and levels of toxins present. Alternariol, alternariol monomethyl ether and tenuazonic acid were produced at 2,5 and 10 mg levels in this culture. This culture was highly cytotoxic and also killed brine shrimp larvae. The pattern of spots obtained by TLC analysis of culture 18 was different from other isolates and almost none of the recognised *Alternaria* mycotoxins were detected but this sample was also toxic in both types of boiassay which suggests that other toxic metabolites were present. Several other isolates had some degree of bioactivity which is difficult to correlate with toxins found.

It is clear that the incidence of mycotoxins at harvest in 1990 was low. However the warm, dry weather persisting during most of the pre-harvest period would not encourage the development and spread of moulds. The question of what the situation would be in a wet Summer remains unanswered. Data from other European studies suggest that incidence and levels might be high. An investigation of mycotoxins under these conditions is recommended as high priority should the opportunity arise.

Table 20 *Alternaria* mycotoxins found in isolate cultures obtained from England and Wales (1990)

Culture no.	µg/culture						
	ATX-I	AOH	ATX-II	AME	ANE	I-ANE	TA
1	50	66	0	0	0	0	0
2*							
3	50	74	0	0	0	0	0
4*							
5	150	50	0	68	0	0	0
6*							
7	500	0	0	0	0	0	0
8	50	7	0	0	0	0	0
9*							
10*							
11	50	9	0	0	0	0	0
12	50	30	0	0	0	0	0
13*							
14	0	2260	310	4900	180	72	10000
15	25	288	0	0	1	0.3	0
16*							
17	25	14	0	9	0	0	0
18	25	4	0	0	0	0	0
19	0	0	0	0	0	0	0
20	0	6	0	0	0	0	0
A. alt.	620	182	280	107	142	42	0
medium control	0	0	0	0	0	0	0

* not examined

Key:

ATX-I - Altertoxin
AOH - Alternariol
ATX- II - Altertoxin II
AME - Alternariol methyl ether
ANE - Altenuene
I-ANE - Iso-altenuene
TA - Tenuazonic acid

three 10 ml volumes of chloroform, which were collected, dried and resuspended for TLC as above (extract II).

Small plugs of the *Fusarium* cultures were extracted with chloroform and examined on TLC plates for toxins.

v) Cytotoxicity tests

Chloroform extracts prepared for TLC were evaporated to dryness under a stream of nitrogen and resuspended in an equivalent volume of ethyl acetate. Extracts were tested for cytotoxicity using HEp2, McCoy, Vero and BHK mammalian cell lines (Flow Laboratories). 200 ul of cell suspension (10^5 cells per ml) were inoculated into each well of a microtitre plate and incubated for 24 hours at 37°C in humidified air containing 5% CO_2. Extracts of the wheats and fungi (1 ul) were placed in triplicate wells with mycotoxin standards as controls. The inoculated cells were incubated for 24 hours before fixing with ethanol and staining with 10% Giemsa stain (Gurr's improved R66, BDH). The cells were examined microscopically and assessed for damage using a scale of 0-4 (1, 25% cell death; 2, 50% cell death; 3, 75% cell death; 4, 100% cell death) (Robb & Norval, 1983).

5.7 Results

i) Fungal isolations

Fusaria were found on most of the wheat samples (Table 21). Twenty four isolates were obtained from the plates including 8 *F. culmorum*, 6 *F. poae*, 2 each of *F. moniliforme, F. tricinctum* and *F. xylarioides* and one each of *F. sporotrichioides, F. oxysporum, F. anthophilum* and *F. heterosporum.*

ii) Mycotoxin analysis

The wheat samples and *Fusarium* isolates were analysed for the following mycotoxins, zearalenone, moniliformin, T-2 toxin, diacetoxyscirpenol, deoxynivalenol, 3-acetyl deoxynivalenol and nivalenol. Within the limitations of our methods, none of these toxins was found in the wheat samples. All the *Fusarium poae* isolates tested produced diacetoxyscirpenol; none of the *F. culmorum* or *F. heterosporum* isolates produced this toxin.

Ringers solution containing 0.2% peptone. The flasks were incubated on a shaker for 21 days at 21°C in a lighted incubator. Nineteen isolates of *Alternaria* were grown in the same way.

iii) Mycotoxin extraction

Wheat samples and the fungal isolates were extracted using the Patterson and Roberts method (Patterson and Roberts, 1979) without the iso-octane (defatting) clean up stage and without dialysis. A flow diagram of the method is shown in Appendix 5.

Samples of wheat (25 g) were ground and extracted with 100 ml acetonitrile : 4% aqueous potassium chloride solution (90:10) by shaking for 30 minutes. The extract (50 ml) was mixed with 20 ml 0.1 M sodium bicarbonate and then extracted with 25 ml chloroform followed by three 10 ml volumes. The chloroform extracts were collected and filtered. No dialysis step was carried out, but the pooled chloroform extracts were evaporated to dryness and resuspended in 0.1 ml chloroform for thin layer chromatography (TLC) analysis (Extract I). The aqueous layer remaining was acidified with 2.5 ml 1N HCl and extracted with three 10 ml volumes of chloroform, which were collected, dried and resuspended as above (extract II).

Fungal cultures were extracted using the same procedure, although each culture was homogenised in a stomacher for 2 minutes and 50 ml of the homogenate were extracted with an equal volume of acetonitrile:potassium chloride (90:10).

iv) TLC analysis

Sample extracts (5 ul) were spotted on TLC plates together with mycotoxin standard solutions. For detection of *Fusarium* toxins, deoxynivalenol, 3-acetyldeoxynivalenol, nivalenol, zearalenone, 15-acetoxy-3,4-scirpendiol, diacetoxyscirpenol, neosolaniol, T2 and T2 triol were used as standards. For *Alternaria* toxins, alternariol, alternariol methyl ether and tentoxin were used.

The TLC plates were developed in toluene:ethyl acetate:90% formic acid (TEF, 60:30:10). TLC plates of extracts from *Fusarium* wheats or *Fusarium* isolates were sprayed with vanillin and charred. Plates for *Alternaria* toxins were viewed unsprayed. For two way TLC, plates were developed in chloroform:methanol (93:7) followed by TEF.

v) Cytotoxicity tests

Chloroform extracts prepared for TLC were evaporated to dryness under a stream of nitrogen and resuspended in an equivalent volume of ethyl acetate. Extracts were tested for affected by the extracts, sample A15 was toxic (score of 4) and sample A1 caused 50% cell death.

iv) Mycotoxin analysis (fungi)

An isolate of *F. poae* from wheat sample 1012 appeared to produce 3-acetyldeoxynivalenol which would be a first record. However this was not confirmed and other *F. poae* isolates will now be checked. Alternariol methyl ether was produced by an *Alternaria* from sample A15. None of the toxins tested for was produced by any other *Fusarium* or *Alternaria* isolates.

v) Cytotoxicity of fungal extracts

The results of cytotoxicity tests with extracts from the fungi are shown in Tables 26 and 27. Extracts from all except one of the *Fusarium poae* isolates tested were toxic to the HEp2 cells, and two (from 1009 and 1045) were toxic to the Chang cells. The other fusaria tested were not toxic to the cells.

Most of the *Alternaria* isolates were toxic to both the HEp2 and Chang cells apart from four (from samples A8, A12, A12 and A18). The *Alternaria* from sample 1048 was not toxic to the HEp2 cells.

5.11 Discussion

Of the wheat samples examined in the *Fusarium* survey, only one contained a toxin for which we had standards (neosolaniol, sample 1012) and in the *Alternaria* wheats one sample only (1048) had detectable levels of tentoxin. Within the limitations of our methodology we were unable to detect any other *Fusarium* or *Alternaria* toxin.

Approximately 33% of the *Fusarium* wheats and 85% of the *Alternaria* wheats were toxic to the HEp2 cells at levels of 2 and over. This is considered above the normal level for most wheats in this laboratory, which normally have toxicity reactions of less than 2.

Of the *Fusarium* species tested (*F.nivale* was not isolated or tested), *Fusarium poae* isolates were consistently the most toxic.

All *Alternaria* isolates apart from four were toxic to the cells. This shows a different pattern to *Alternaria* isolates from oil seed rape, which showed variable toxicity due to species, temperature and length of incubation (McKenzie, Robb & Lennard, 1988).

Table 21 Incidence of *Fusarium* spp. on wheat grain samples from Scotland (1989)

Sample number	% *Fusarium*	Sample number	% *Fusarium*
1	18	20	36
2	10	21	6
3	38	22	4
4	4	23	14
5	16	242	24
6	20	244	8
7	4	261	34
8	8	284	42
9	18	295	32
10	20	A	0*
11	0	B	15*
12	20	C	10*
13	6	D	12
14	6	E	0
15	20	F	14
16	8	G	20
17	18	H	0**
18	20	I	13.3**
19	38	J	6.7**

* Values from unsterilised seed.

** Values from surface sterilised seed.

Table 23 Incidence of *Alternaria* spp. on wheat grain samples from Scotland (1990)

Sample number	% *Alternaria*	Sample number	% *Alternaria*
A1	52	A14	62
A4	82	A15	54
A8	80	A16	76
A9	38	A18	12
A10	88	A20	72
A11	76	A23	40
A12	50		

Table 24 (continued)

Sample number	Extract I or II	Cytotoxicity (average of 3 wells) [1]		
		HEp2 cells	Vero cells	Chang cells
1012	(I)	0.5		1.5
	(II)	0		
1013	(I)	-		
	(II)	0.5		
1014	(I)	1		0
	(II)	2.5		
1016	(I)	0.5		1
	(II)	2.5		
1017	(I)	-		
	(II)	2		
1018	(I)	2.5	0	0
	(II)	1.5		
1019	(I)	1	0.5	0
	(II)	1.5		
1020	(I)	0	0	0
	(II)	1.5		
1021	(I)	0	0	0
	(II)	0.5		
1022	(I)	1	0	0
	(II)	2.5		
1023	(I)	3	0.5	0.5
	(II)	2.5		

[1] Scale from 0-4 (1, 25% cell death; 2, 50% cell death; 3, 75% cell death; 4, 100% cell death).

Table 25 Cytotoxicity reactions of extracts from *Alternaria* wheat grain samples from Scotland (1990)

Sample number	Extract I or II	Cytotoxicity reaction [1]		% *Alternaria*
		HEp2 cells	Chang cells	
A1	(I)	1	2	52
	(II)	2		
A4	(I)	3	0.5	82
	(II)	2.5		
A8	(I)	3	0	80
	(II)	2.5		
A9	(I)	2.5	0	38
	(II)	2.5		
A10	(I)	2.5	0	88
	(II)	2		
A11	(I)	3	0	76
	(II)	2		
A12	(I)	0.5	0	50
	(II)	1.5		
A14	(I)	1.5	0	62
	(II)	2.5		
A15	(I)	3	4	54
	(II)	1.5		
A16	(I)	3	0	76
	(II)	1.5		
A18	(I)	0	0	12
	(II)	1.5		
A20	(I)	3.5	0	72
	(II)	2.5		
A23	(I)	1.5	0	40
	(II)	3		

[1] Scale from 0-4 (1, 25% cell death; 2, 50% cell death; 3, 75% cell death; 4, 100% cell death)

Table 27 Cytotoxicity reactions of *Alternaria* isolates obtained from wheat grain samples from Scotland (1990)

Alternaria species	Wheat sample number	Cytotoxicity reaction [1] HEp2 cells	Chang cells
Alternaria alternata	A8	3.5	4
Alternaria sp.	A8	3	2.5
Alternaria alternata	A8	0.5	0
Alternaria sp.	A9	2.5	2
Alternaria sp.	A10	3.5	3.5
Alternaria sp.	A10	3	3.5
Alternaria sp.	A11	2.5	2.5
Alternaria sp.	A12	1.5	1
Alternaria sp.	A12	0	0
Alternaria sp.	A14	3	3
Alternaria alternata	A15	3	3
Alternaria sp.	A15	2.5	3
Alternaria sp.	A16	2.5	2.5
Alternaria sp.	A18	3.5	3.5
Alternaria sp.	A18	0.5	1
Alternaria sp.	A18	3.5	3.5
Alternaria sp.	A23	3.5	3.5
Alternaria sp.	A23	3	3
Alternaria sp.	1048	0.5	3.5

[1] Scale from 0-4 (1, 25% cell death; 2, 50% cell death; 3, 75% cell death; 4, 100% cell death)

Details of fungicides tested against *Fusarium* spp. including site of action and approved uses

Trade name	Active ingredient	Site of action	Approved usage (cereals/turf)
Benlate	benomyl	microtubule formation	eyespot, *Rhynchosporium*, *Fusarium* patch in turf
Sportak 45	prochloraz	ergosterol biosynthesis	eyespot, glume blotch, powdery mildew
Rovral	iprodione	enzyme inhibition	glume blotch, *Fusarium* patch in turf
Exp. sample	flusilazole	ergosterol biosynthesis	-
Exp. triazole	-	ergosterol biosynthesis	-

Exp. = Experimental

6.4 Results

Figures 1-5 show the average dosage-response curves for *F. nivale* and *F. culmorum* to benomyl, prochloraz, iprodione, flusilazole and an experimental triazole. EC50 values for each fungicide were estimated from the graphs (Table 29). The graphs illustrate the gradual effect of increasing concentration of the ergosterol biosynthesis inhibitors, which affect a single step in an enzyme pathway, compared to the 'all or nothing' effect of site specific inhibitors such as benomyl and iprodione. The gradual response of *F. nivale* to benomyl is an indication of resistance to the fungicide.

EC50 values show a significant difference between *F. nivale* and *F. culmorum* in sensitivity to benomyl and iprodione. Isolates of *F. nivale* were significantly less sensitive to benomyl and significantly more sensitive to iprodione than *F. culmorum*. Prochloraz was found to be the most effective fungicide tested against *Fusarium* spp. The experimental triazole and flusilazole showed similar levels of activity both being slightly more effective against *F. culmorum* than *F. nivale*.

Average sensitivity values for isolates of 5 species of *Fusarium* in 1989 are shown in Tables 30 and 31. In 1990, three species were tested (Tables 32 & 33). In 1989 and 1990 all isolates

(Gilmour, 1991). The sensitivity of isolates of *F. nivale*, *F. avenaceum* and *F. culmorum* was investigated in a survey undertaken in 1986 at growth stage 73-75 (Locke *et al.*, 1987). Of the 581 isolates identified as *F. nivale*, 92% were found to be resistant to benomyl at 20 ppm. None of the isolates of other species were resistant. Resistance was defined as the ability of the isolates to grow in the presence of a fungicide at a particular concentration. If this definition was applied to the present study then 78% of isolates of *F. nivale* could be termed resistant in 1989 and 87% of isolates in 1990. The measured resistance levels of isolates obtained at GS31 may be expected to be lower than resistance levels of isolates obtained at GS 73-75, as isolates at GS 31 will have been less exposed to selection pressure of repeated fungicide sprays. However comparison of the growth of *F. culmorum* and *F. avenaceum* isolates, obtained from plots previously treated with carbendazim or prochloraz or left untreated, on agar containing a range of concentrations of carbendazim or prochloraz, showed that there was no influence of previous fungicide treatment on the growth of either species and no evidence of decreased sensitivity to either carbendazim or prochloraz.

Gilmour (1991) stated "the term resistance should be used only where there has been an inheritable, stable decrease in sensitivity of a pathogen to a fungicide as measured in some appropriate test. Ideally the term should be reserved for those situations where the decrease in sensitivity has resulted in a loss of field performance". Resistance detected in *in vitro* tests is not necessarily related to resistance in the field and therefore isolates showing lowered sensitivity in tests carried out in this survey have not been termed resistant as loss of field performance has not been demonstrated.

Isolates of *F. nivale* showed a wide range of sensitivity to the triazole or EBI fungicides tested. Growth of benomyl-insensitive isolates was effectively inhibited. The range of sensitivities found may indicate the presence or development of insensitivity to EBI fungicides and further examination of responses to these fungicides is recommended. In the United States (Watkins *et al.*, 1987) and Belgium (Maraite *et al.*, 1988) experience has indicated that some ergosterol biosynthesis inhibitor (EBI) fungicides are likely to be useful in control of *Fusarium* patch caused by *F. nivale*. However, Prince *et al.* (1989) report that repeated use of these fungicides could lead to resistance problems. The difference in sensitivity to different triazole fungicides demonstrated in this survey indicates that further fungicides of this group should be tested in order to find the most effective molecule for control of *Fusarium* diseases.

Isolates of *F. nivale* isolated from *Fusarium* patch symptoms on golf greens in New Zealand (Pennucci *et al.*, 1990) and America (Chastagner & Vassey, 1982) have been shown to be insensitive to dicarboximide fungicides *in vitro* and resistant to treatment in the field. Resistant isolates were able to grow on PDA agar amended with iprodione at 10 mg/l (Chastagner & Vassey, 1982) and all dicarboximide-resistant isolates obtained in New

FIG. 1 DOSE RESPONSE CURVE FOR BENOMYL

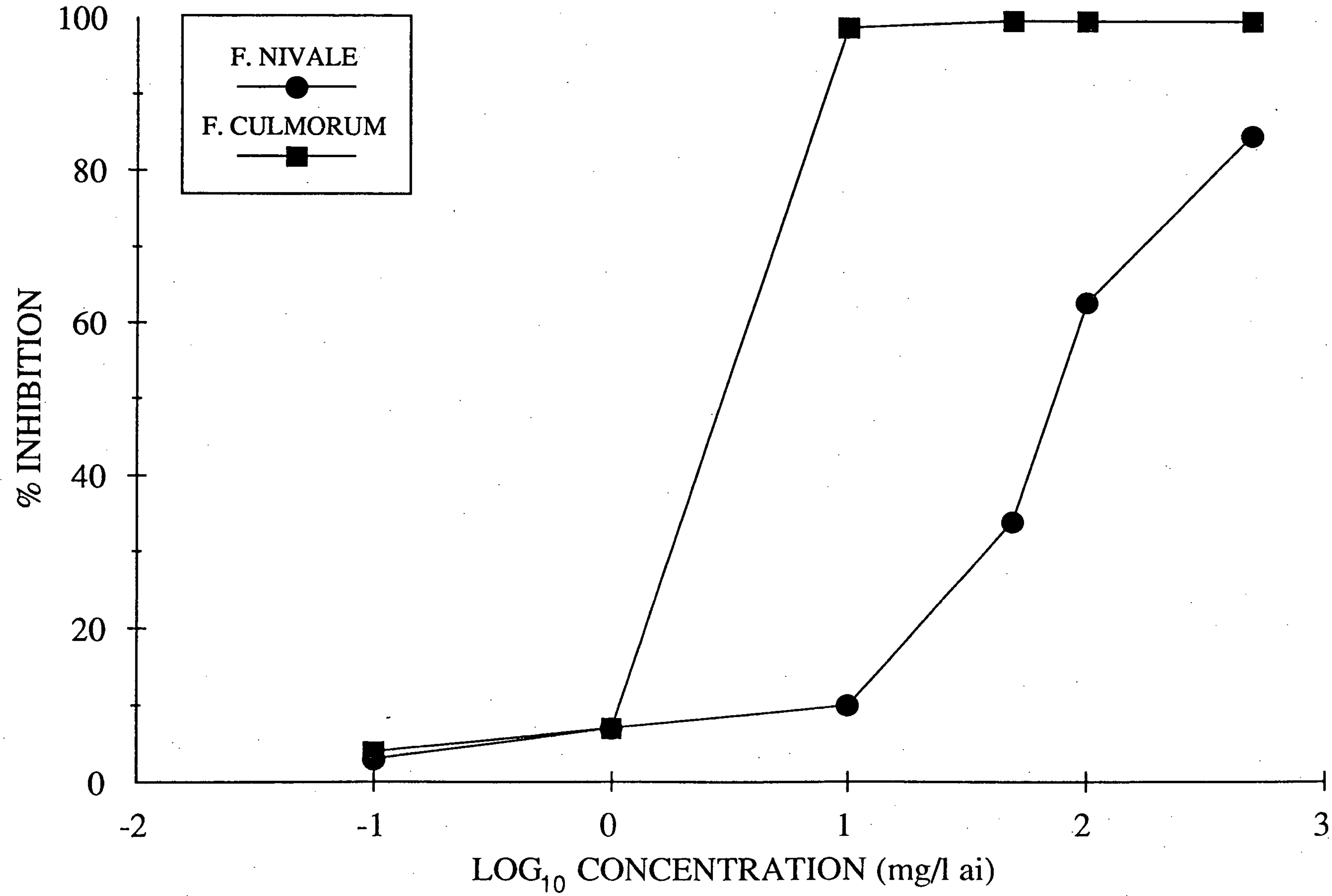


FIG. 3 DOSE RESPONSE CURVE FOR IPRODIONE

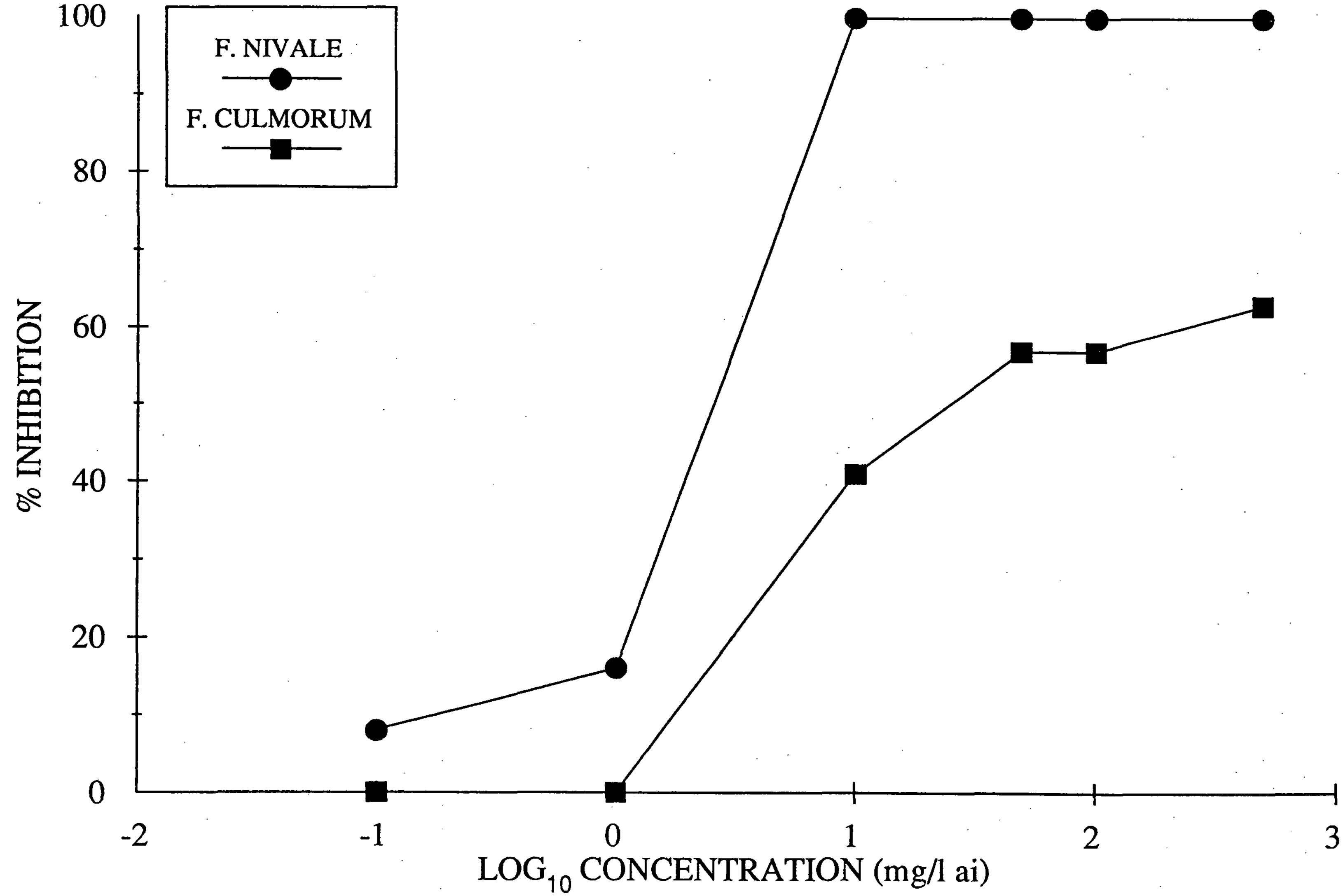


FIG. 5 DOSE RESPONSE CURVE FOR EXPERIMENTAL TRIAZOLE

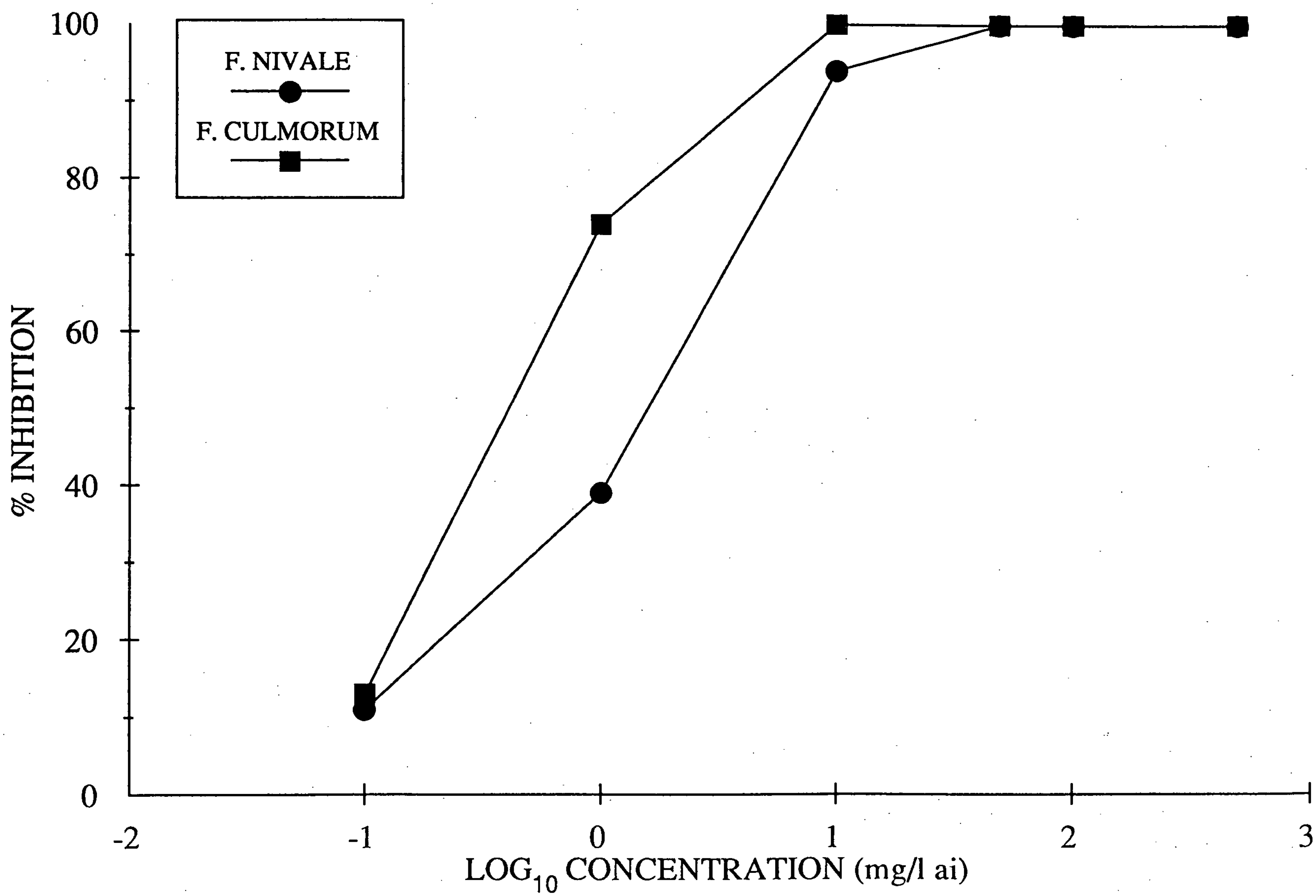


Table 29 EC50 values for fungicides tested against *Fusarium* species

Fungicide	*Fusarium* species	EC50 (mg/l ai)*
benomyl	*F. nivale*	71.8
	F. culmorum	2.93
prochloraz	*F. nivale*	< 0.1
	F. culmorum	< 0.1
iprodione	*F. nivale*	2.5
	F. culmorum	25.1
flusilazole	*F. nivale*	1.85
	F. culmorum	0.37
exp. triazole	*F. nivale*	1.58
	F. culmorum	0.40

* mean of 10 isolates obtained from samples collected at sites throughout England, Wales and Scotland.

EC50 = effective concentration which reduces growth by 50%.

Table 31 Sensitivity of *Fusarium* species to fungicides after 10 days incubation

		Mean inhibition as % of control				
Fungicide	conc (mg/l)	*F. nivale*	*F. culmorum*	*F. avenaceum*	*F. graminearum*	*F. sporotrichoides*
benomyl	20	66.6	100.0	100.0	100.0	100.0
	5	61.9	93.8	75.7	73.0	83.9
prochloraz	2	97.3	79.4	85.8	89.6	46.6
	0.05	55.8	17.9	36.7	41.2	38.9
fluzilazole	1.6	95.7	88.4	68.0	54.2	100.0
	16	52.3	61.0	40.1	33.3	89.6
iprodione	5	55.4	5.2	29.6	28.2	49.1
Total isolates tested		32 (45)*	10	6	7	4

* 13 extra isolates were tested against benomyl at 20 and 5 mg/l.

Table 33 Sensitivity of *Fusarium* species to fungicides after 10 days incubation (1990)

Fungicide	Conc (mg/l ai)	Mean inhibition as % of control *F. nivale*	*F. culmorum*	*F. avenaceum*
benomyl	20.0	15.6	100.0	100.0
	5.0	13.2	54.3	74.1
prochloraz	2.0	86.9	82.0	82.1
	0.05	33.6	15.2	22.6
fluzilazole	16.0	85.2	90.4	53.9
	1.6	41.3	50.7	25.1
iprodione	5.0	61.2	6.5	14.2
exp. triazole	10.0	77.7	93.2	-
	1.0	13.4	53.0	-
Total isolates tested		31	21	4

Figure 8. Sensitivity of *F. nivale* to prochloraz (0.05ppm)
1989

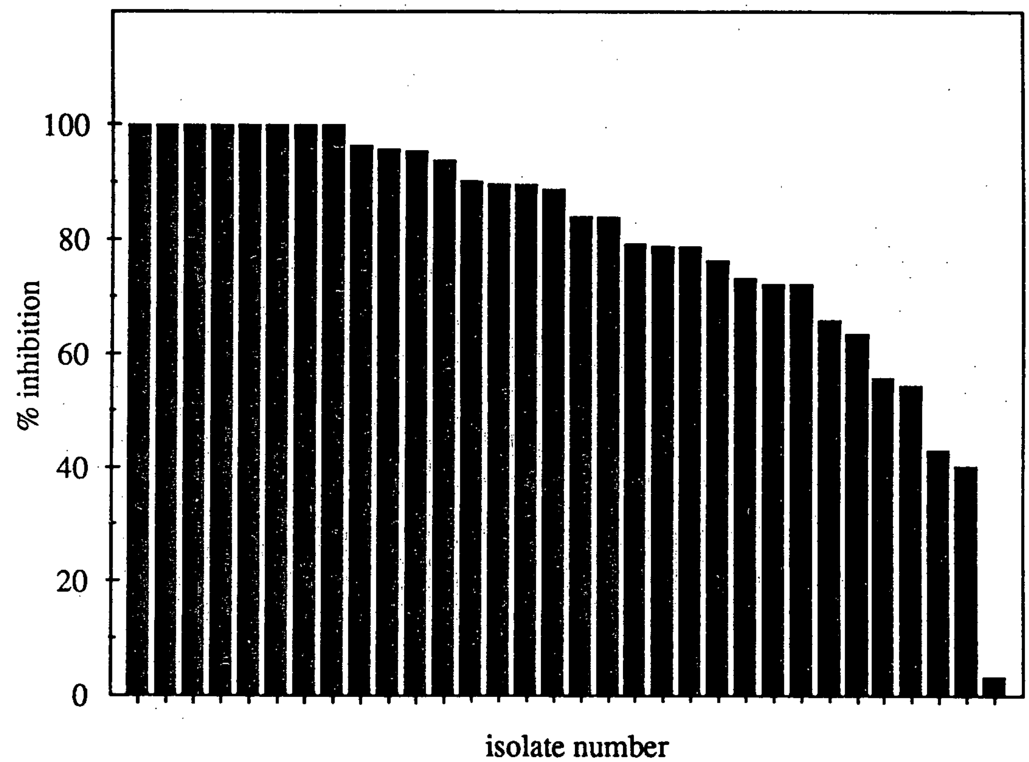


Figure 9. Sensitivity of *F. culmorum* to prochloraz (0.05ppm)
1989

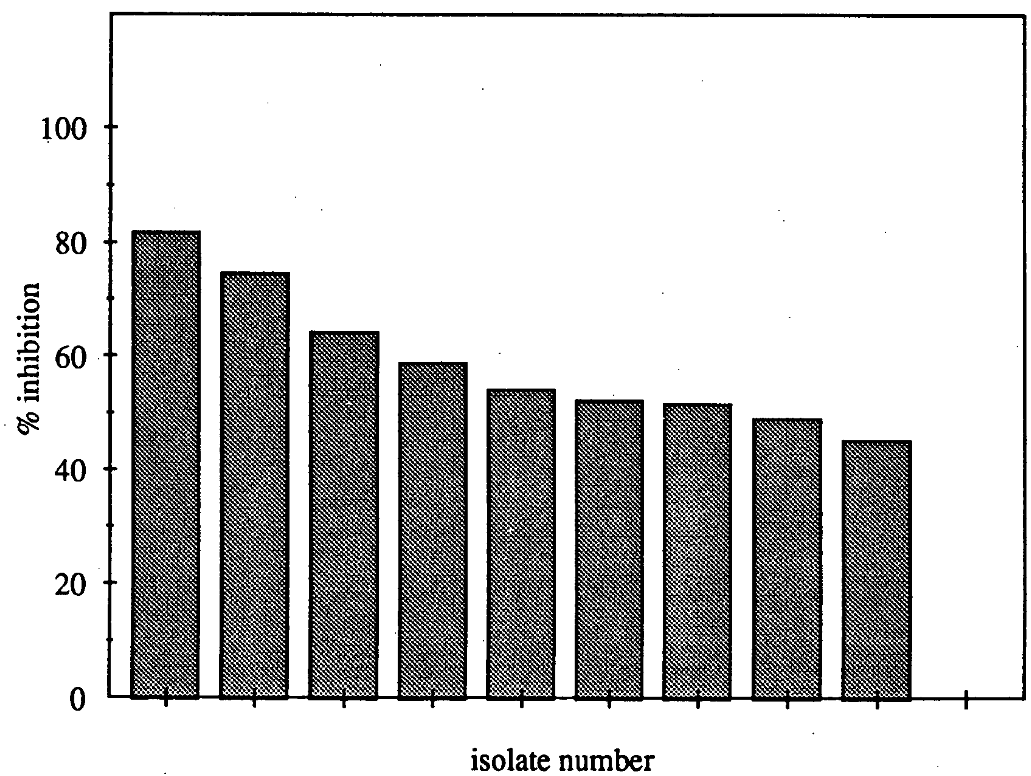


Figure 12. Sensitivity of *F. nivale* to flusilazole (1.6ppm)
1989

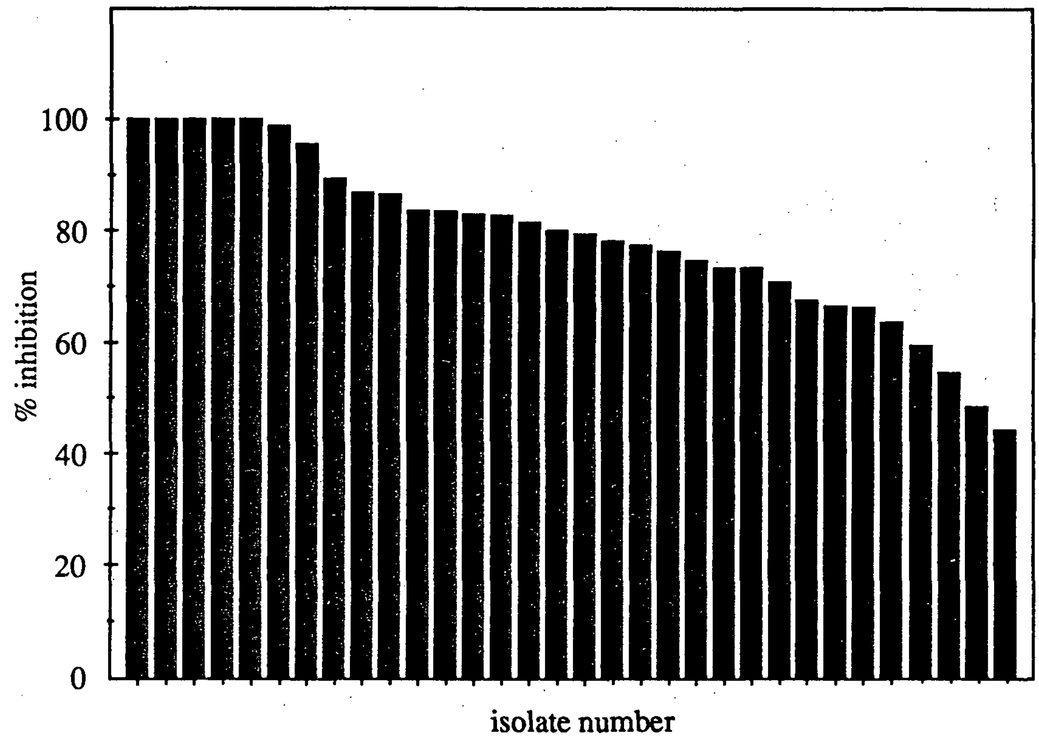


Figure 13. Sensitivity of *F. culmorum* to flusilazole (1.6ppm)
1989

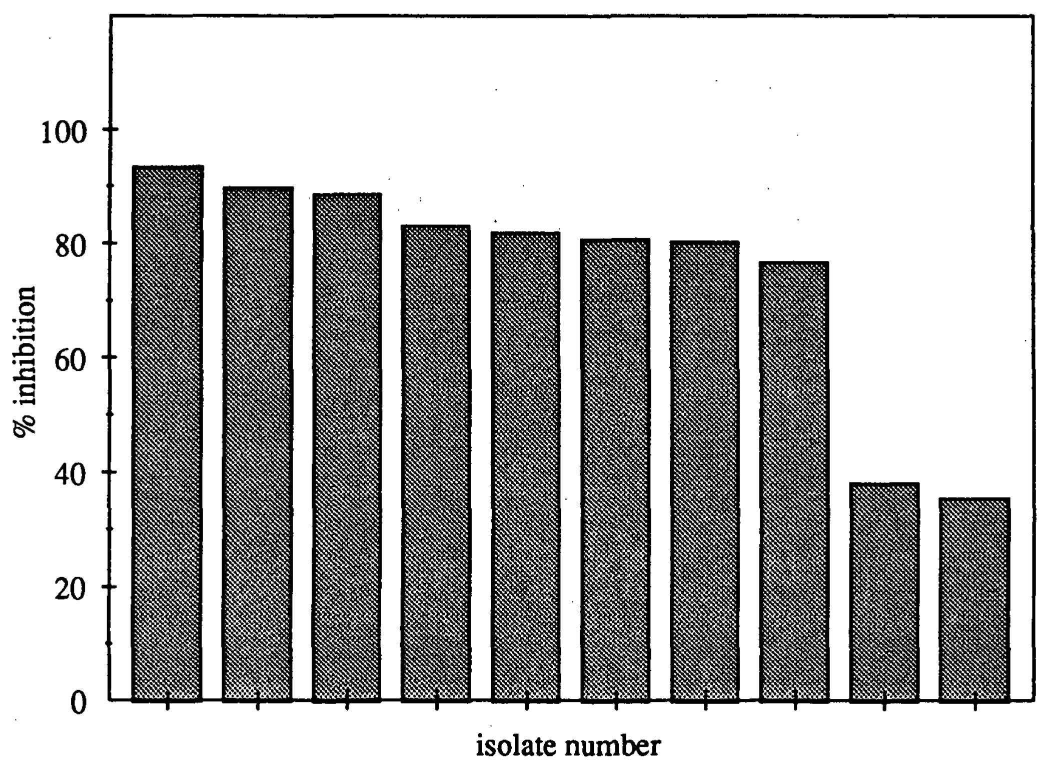


Figure 16. Sensitivity of *F. nivale* to prochloraz (0.05ppm)
1990

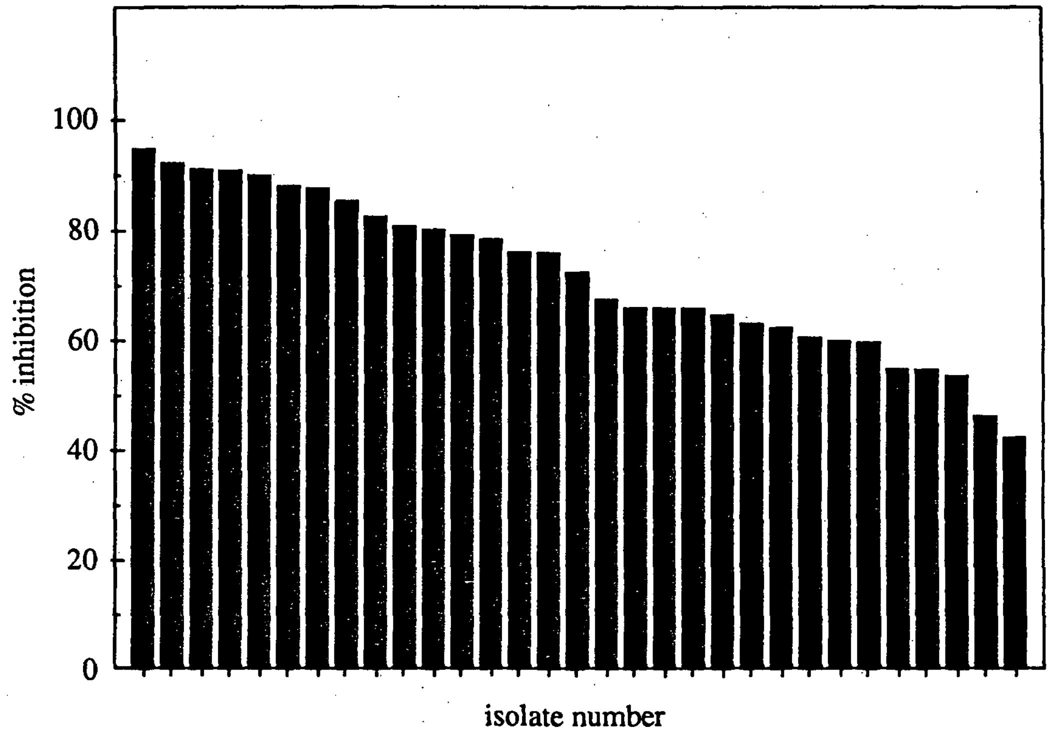


Figure 17. Sensitivity of *F. culmorum* to prochloraz (0.05ppm)
1990

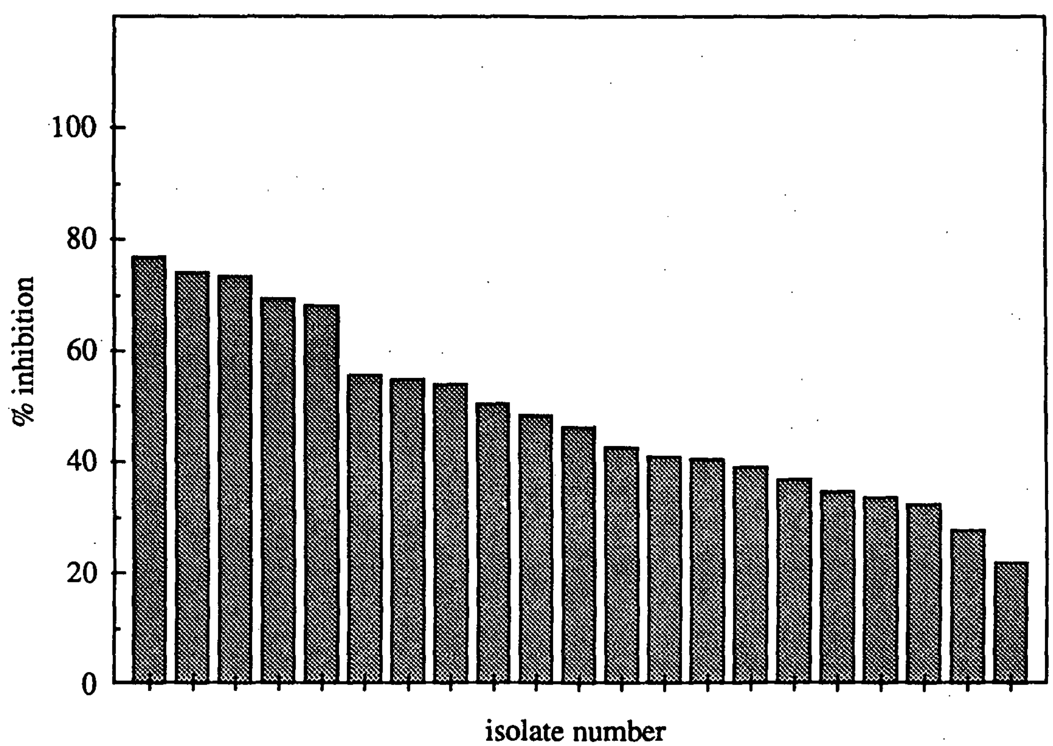


Figure 20. Sensitivity of *F. nivale* to flusilazole (1.6ppm)

1990

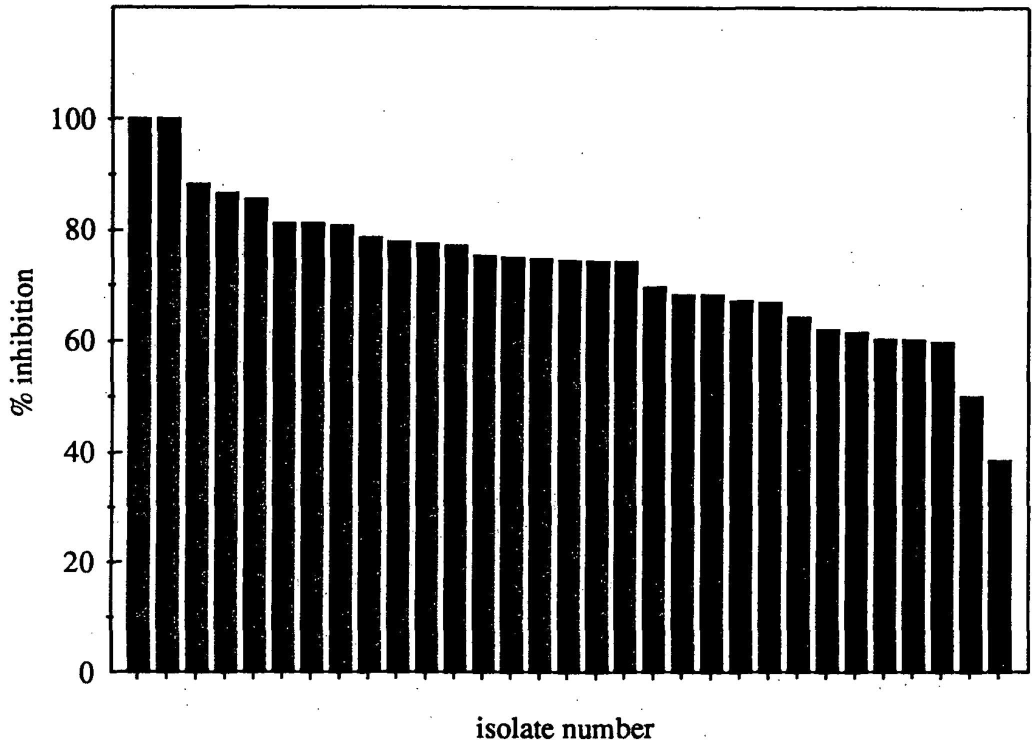


Figure 21. Sensitivity of *F. culmorum* to flusilazole (1.6ppm)

1990

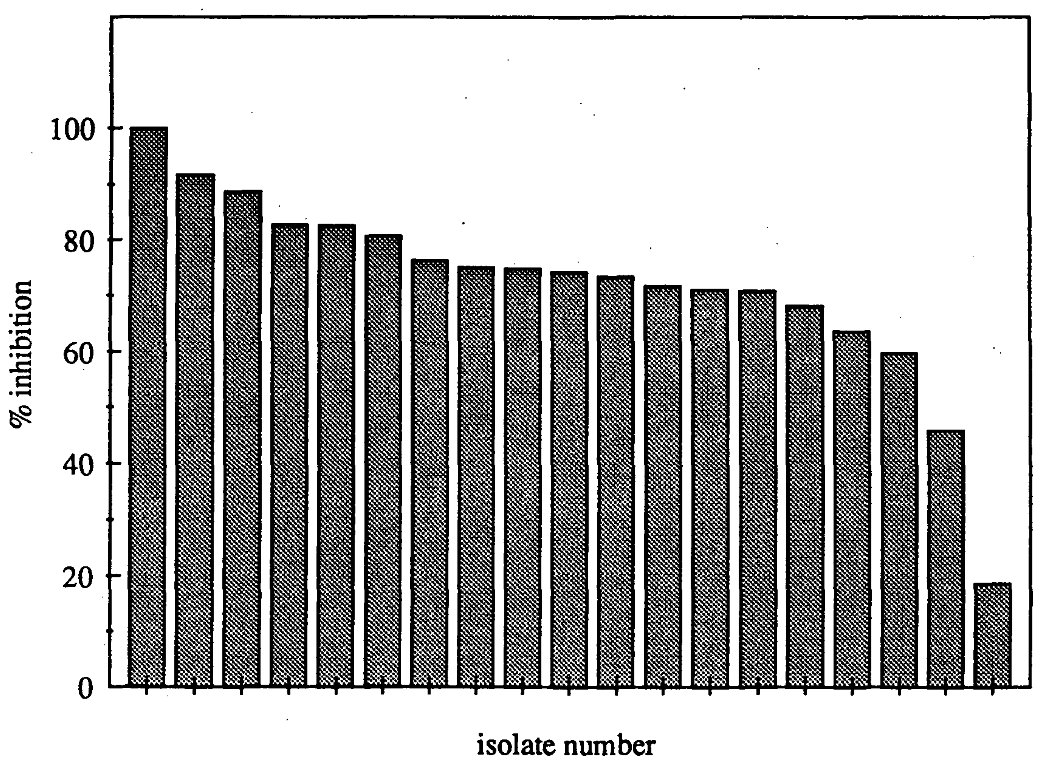


Figure 24. Sensitivity of *F. avenaceum* to selected fungicides

1989

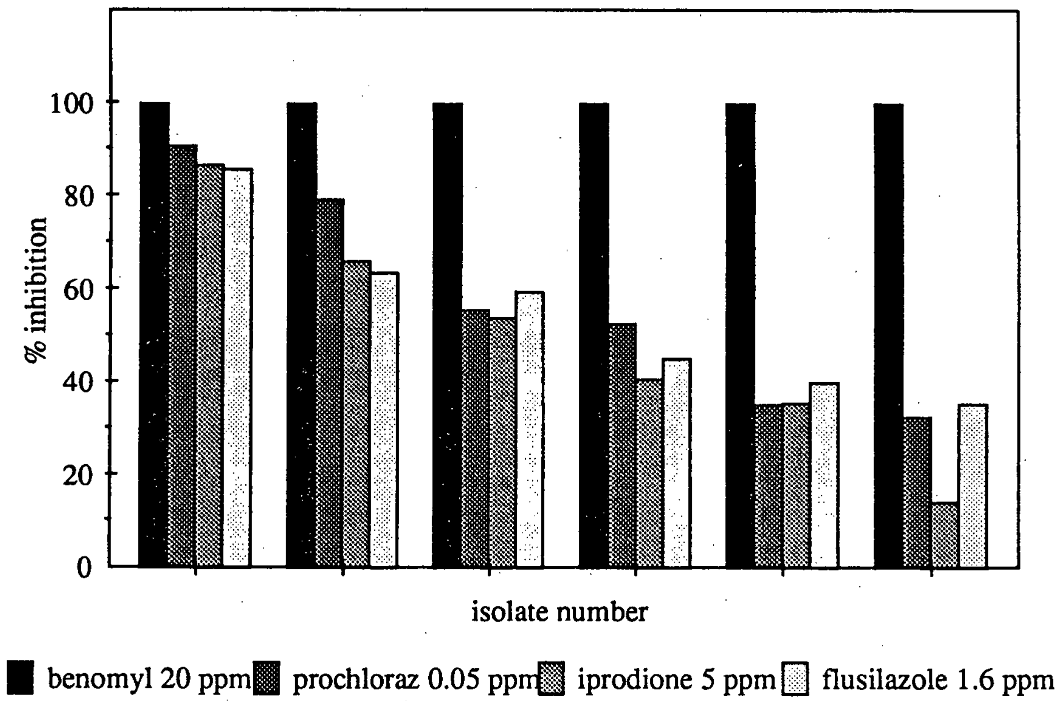
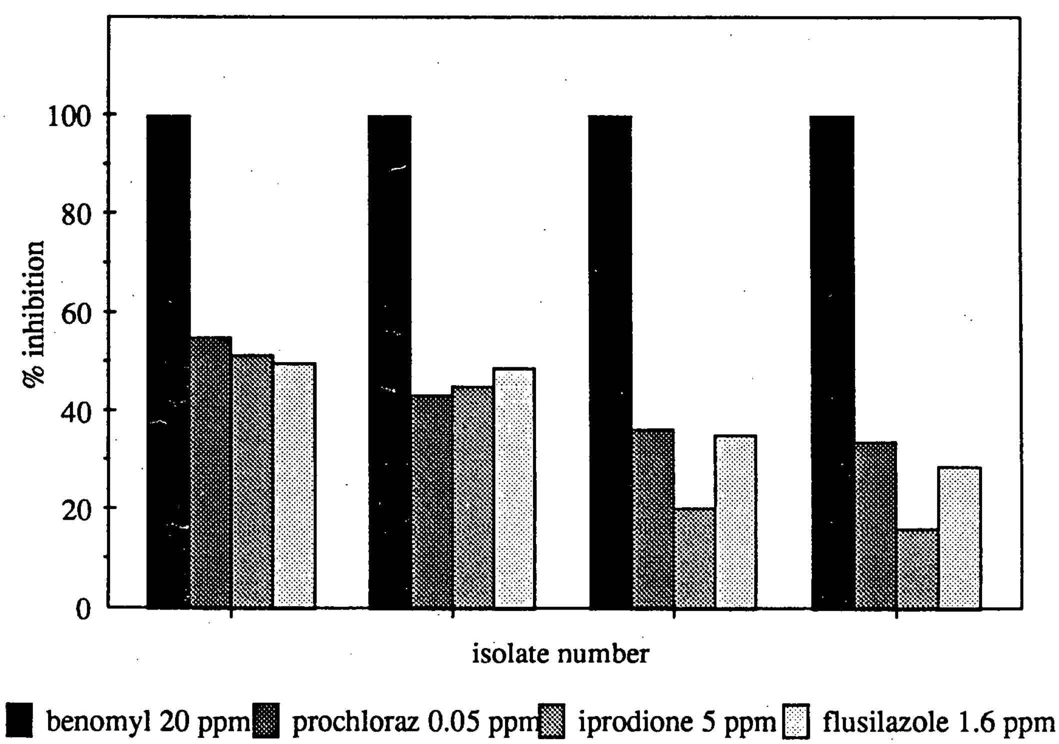


Figure 25. Sensitivity of *F. avenaceum* to selected fungicides

1990



tested. However data from ADAS Winter Wheat Disease Surveys show no relationship between use of prochloraz at GS 31 and incidence of *Fusarium* symptoms at GS 71-73 and it may be that a related fungicide or a cocktail of fungicides could prove more effective in the field. The disparity between the effectiveness of fungicides in laboratory sensitivity tests and in the field suggests that further work on the rate and timing of fungicide applications is required to optimise the efficacy of fungicide sprays against *Fusarium*.

Fusarium infection of the ears at GS 73 was dominated by *F. poae* in both 1989 and 1990. The scarcity of fusarium ear blight caused by *F. culmorum* was probably due to the dry conditions during early summer in both years. However in harvested grain *F. culmorum* was the predominant species in samples from England and Wales in 1989 and equally as prevalent as *F. poae* in 1990. *F. nivale* was also common in grain samples particularly in Scotland. Again this may reflect the effect of weather conditions. The potential of *F. nivale* to cause severe pre-emergence death (Hewett, 1983) and the imminent withdrawal of organomercury as a seed treatment underlines the urgency for monitoring seed-borne infection.

The survey of blackpoint in grain in 1990 showed that only very low levels were present, again highlighting the need for further evaluation in a wet season.

No mycotoxins were detected in grain samples from Scotland in 1989, and only *F. poae* isolates produced toxins under the particular assay conditions used as part of this survey. In 1990 only one sample from Scotland in the *Fusarium* survey contained a toxin. One grain sample from the 13 fields in the *Alternaria* survey in Scotland had a detectable level of tentoxin. Of the fungal isolates tested, only one *F. poae* isolate and one *Alternaria* isolate produced a mycotoxin. However, the *Alternaria* isolates were extremely toxic to the tissue cultures.

Fusarium mycotoxins were detected in approximately 25% of the wheat grain samples examined from England and Wales in 1990. Those samples found to be contaminated often contained two or more mycotoxins with nivalenol and deoxynivalenol frequently occurring together. A small number of samples were exceptional in containing high levels of several trichothecenes. These were among the ten grain samples received in poor condition from one ADAS region due to a delay in transit. However, while not reflecting the situation at harvest, these samples serve to illustrate the dangers which can arise if grain is not stored correctly. Most *Fusarium* isolates obtained from the ADAS samples were cytotoxic, but, as with the Scottish samples, they produced very few mycotoxins.

No *Alternaria* mycotoxins were found in any of the bread-making wheat samples received from the ADAS regions. However, the limits of detection of the methods developed were not as sensitive as those used for detecting trichothecenes. In contrast to the results from

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APPENDIX 2 EXTRACTION AND CLEAN-UP PROCEDURE FOR THE DETECTION OF MYCOTOXINS AND BIOLOGICAL ACTIVITY IN WHEAT

<u>FUSARIUM</u>

60 g wheat

| extract with 216 ml acetonitrile + 24 ml 4% aqueous KCl

filtrate

|

150ml

|

trichothecenes
zearalenone/-ol
cytotoxicity
brine shrimp

|

100ml filtrate

|

20 ml	30 ml	50 ml
charcoal-alumina column clean-up	partition into water and dichloromethane	partition against chloroform/sodium bicarbonate
TFA derivatisation	GPC clean-up	Re-extract with HCl and chloroform
GC/MS detection	HPLC analysis	recombine with chloroform solution
TRICHOTHECENES	ZEARALENONE	CYTOTOXICITY / BRINE SHRIMP

APPENDIX 3 METHODS DEVELOPED FOR DETERMINATION OF ALTERNARIA TOXINS

The scientific literature contains very little information on validated methodology for the determination of *Alternaria* mycotoxins in wheat. Methods were developed for the six mycotoxins analysed for in this work and a TLC method for determination of tenuazonic acid was set up. Once suitable extraction and clean-up procedures had been developed and tested, the method was validated for each mycotoxin by spiking uncontaminated wheat with known levels of mycotoxins. Recoveries were determined and these fell between 70% and 90%, depending on the toxin. It is planned to present full details of the methods and validation in a paper to be submitted for publication. A summary of the methods used in this study is given below.

i) altertoxin I,alternariol, altertoxin II and alternariol methyl ether.

A Nucleosil C18, 10cm HPLC column fitted with a guard column and using a mobile phase of acetonitrile: 1% phosphoric acid at a flow rate of 1 ml /minute gave separation of these toxins with retention times of 13, 19, 32 and 67 minutes respectively. Detection was by UV at 340 nm.

ii) altenuene and iso-altenuene.

The column used was a Spherisorb ODS (5u) analytical column+guard column eluting with methanol:water, 45:55. Detection was by fluorescence, excitation 280 nm, emission 475 nm. The mycotoxins elute at 13.2 and 15.2 minutes respectively.

iii) TLC (cultures only)

TLC for all above mycotoxins was carried out on Silica gel 60 aluminium backed sheets 20x20 cm cut squares. Developing solvents were a) chloroform:acetone, 54:6 and b) toluene: ethyl acetate: formic acid,36:18:6.

iv) TLC for tenuazonic acid (cultures only)

This was carried out on silica gel plates containing fluorescent indicator and treated with tartaric acid. Solvents used were those in iii).

APPENDIX 5 FLOW DIAGRAM OF EXTRACTION METHOD USED FOR WHEAT SAMPLES

25 g wheat

extract with 100 ml acetonitrile:4% aqueous KCl (90:10)

50 ml filtrate

add 20 ml 0.1 M sodium bicarbonate

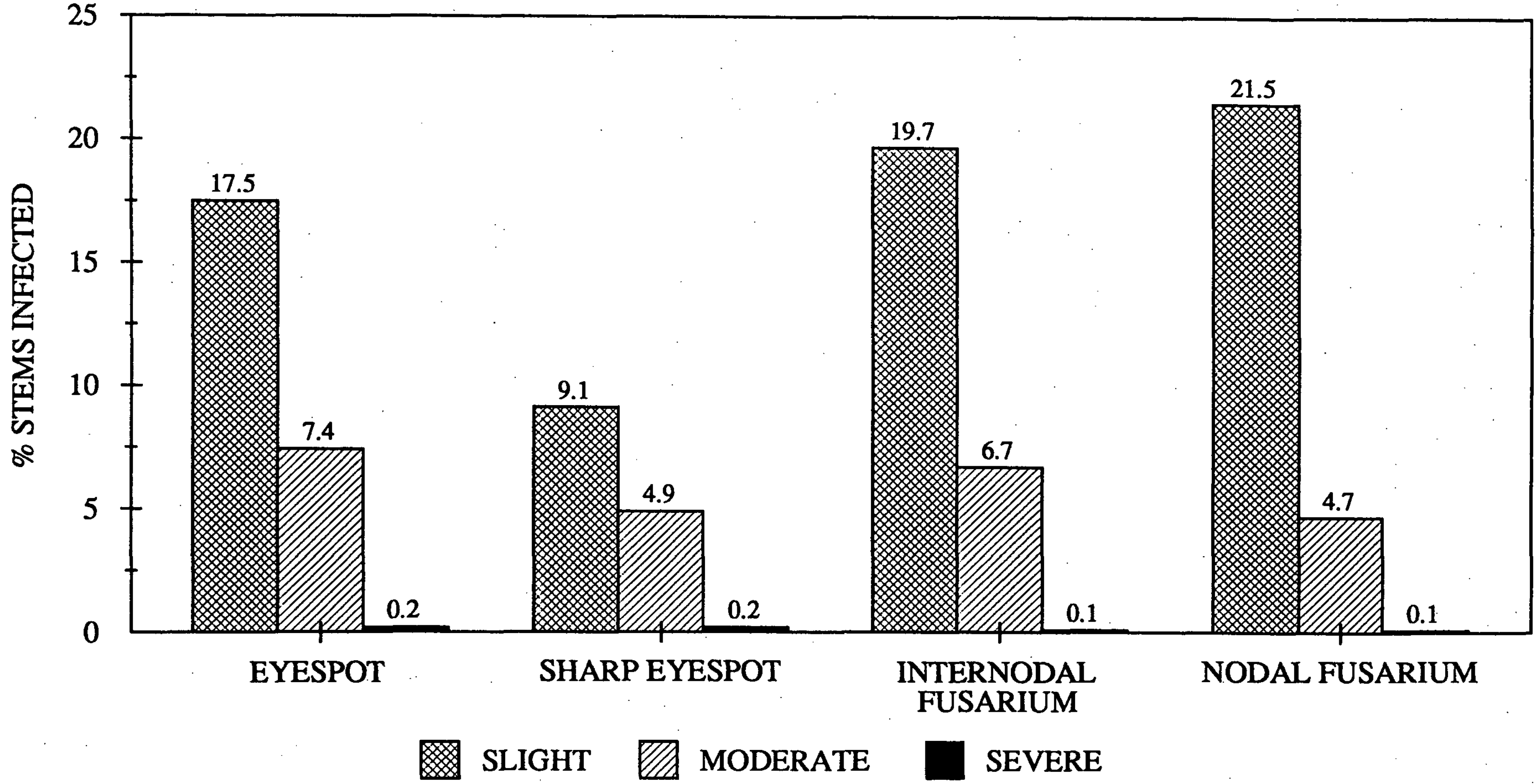
extract with 25 ml chloroform, followed by 3 x 10 ml chloroform

chloroform extract	aqueous phase
filtered and dried down	acidified with 2.5 ml 1N HCl
resuspended in 0.1 ml (EXTRACT I)	extracted with 3 x 10 ml chloroform

chloroform extract dried down and resuspended in 0.1 ml (EXTRACT II)

WINTER WHEAT 1990

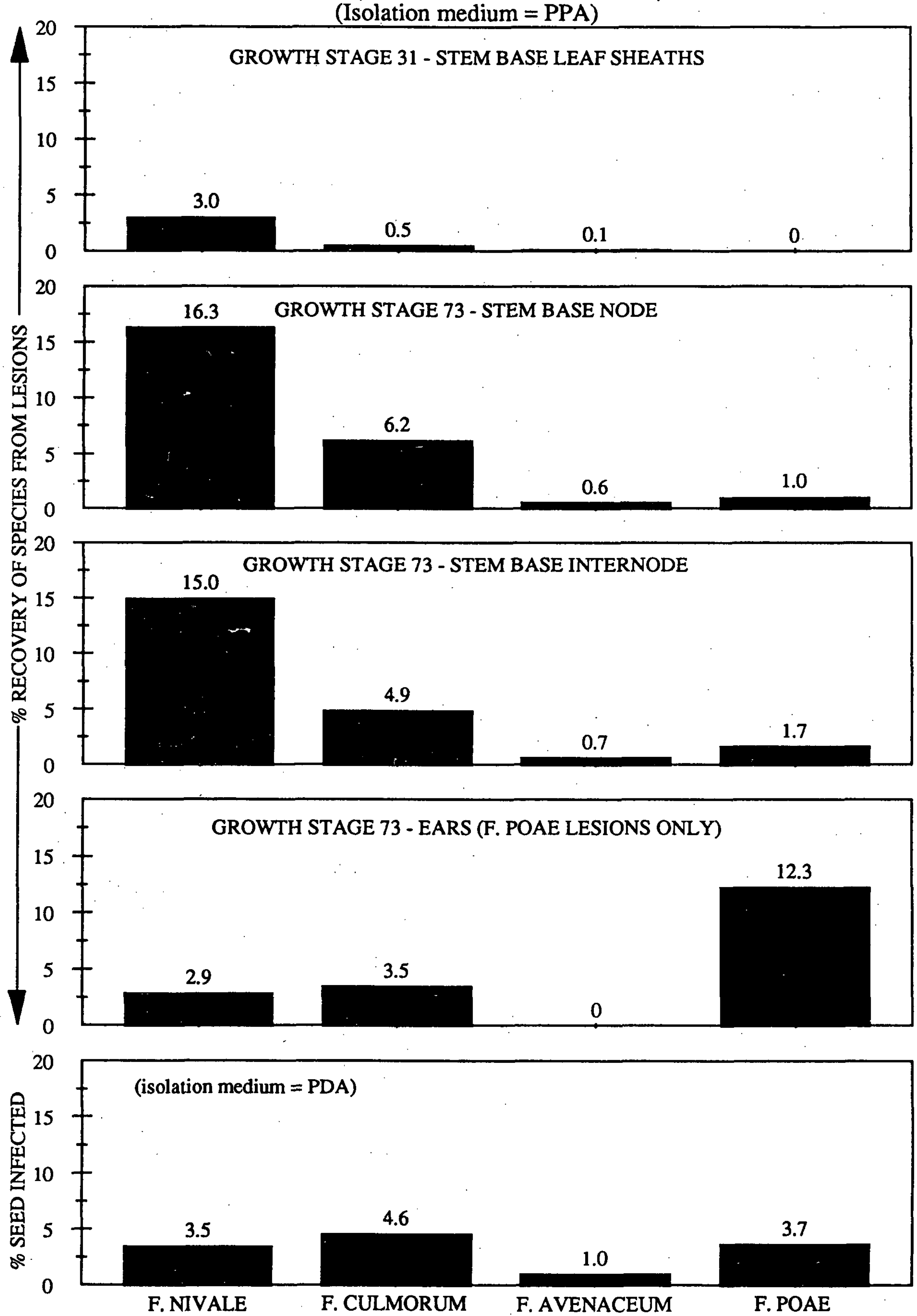
NATIONAL STEM BASE DISEASE LEVELS
(AVERAGE % STEMS INFECTED)



Source: ADAS Winter Wheat Disease Survey

FIG. 27

FIG 29.
INCIDENCE OF FUSARIUM SPECIES ON WINTER WHEAT
IN ENGLAND, SCOTLAND AND WALES, 1989.
(Isolation medium = PPA)



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