Project title	Apple: Studies on *Fusarium* species causing core rots and storage rots
Project number:	TF 203
Project leader:	Dr A M Berrie, East Malling Research
Report:	Final report, 2013
Previous report:	None
Key staff:	Dr A Berrie Dr R Saville Mrs K Lower Miss Eleri Tudor
Location of project:	East Malling Research
Project coordinator:	Giles Cannon, GSR Fruits Ltd, Kent
Date project commenced:	1 April 2012
Date project completed (or expected completion date):	30 June 2013

DISCLAIMER

AHDB, operating through its HDC division seeks to ensure that the information contained within this document is accurate at the time of printing. No warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

[Name]
[Position]
[Organisation]

Signature ..... Date .....

[Name]
[Position]
[Organisation]

Signature ..... Date .....

Report authorised by:

[Name]
[Position]
[Organisation]

Signature ..... Date .....

[Name]
[Position]
[Organisation]

Signature ..... Date .....

CONTENTS

GROWER SUMMARY

Headline

- *Fusarium tricinctum* was identified as the main species of *Fusarium* responsible for mouldy core in UK apples.

Background and expected deliverables

HDC project TF184 (which studied mouldy core) identified *Fusarium* spp. as the main fungal species responsible for core rots in both the varieties Cameo and Bramley. In HDC project TF193, *Fusarium* species were also shown to be responsible for storage rots in Bramley, causing stalk, cheek and eye rots, particularly in longer term stored Bramley, where they could account for around 30% of the rotting (actual losses due to rots 2-10%). Several species of *Fusarium* were isolated from core rots and post-harvest rots, but the actual species were not identified.

Several *Fusarium* species, especially *Fusarium avenaceum*, are also responsible for ear blights in cereals where they produce a range of mycotoxins and cereal products are routinely screened for these to see if they exceed strict EU health limits.

Apple rots caused by *Fusarium* spp. appear to be increasing in incidence in other countries in Europe. Recent investigations on wet core rots on apple in Slovenia (varieties Gloster, Jonagold and Fuji), identified *F. avenaceum* as the main cause of the rots and showed high levels of mycotoxins present in apples with wet core rot (Sorensen *et al*., 2009). Recent advances in *Fusarium* taxonomy based on molecular techniques mean that identification of *Fusarium* species is relatively straight forward. The purpose of this project was to identify the main *Fusarium* species responsible for core rots and post-harvest rots in apples.

Summary of the project and main conclusions

Isolates of *Fusarium* were obtained either from fruit rots collected during apple rot surveys in 2010 / 2011, 2011 / 2012 and 2012/ 2013, or from fruit collected from orchards of the varieties Bramley or Cameo in Kent, Cambridgeshire and Suffolk in August 2012. Rots were isolated on Potato Dextrose Agar. A total of 120 isolates were collected. Species of *Fusarium* were identified by DNA analysis. DNA was extracted from the fungal mycelium and sequenced for a particular region. The resulting sequences were compared to

Fusarium databases available on the internet to identify the species. Almost all *Fusarium* isolates were identified as *F*. *tricinctum* species complex 1-a which is closely related to *F. avenaceum*.

Mouldy core of apple is undetectable until the fruit is cut open or consumed and in the case of processed apple, may never be detected. Further work is required to understand the epidemiology of *Fusarium* in apple orchards to reduce its incidence in the future.

Financial benefits

Apples used for processing and in particular for juice production, are generally routinely checked for the mycotoxin patulin produced by certain species of *Penicillium*. *Fusarium* species have been shown to cause core rots and post-harvest rots in apples, particularly Cameo and Bramley. In this project, the *Fusarium* species in apple has been identified as *F*. *tricinctum*. This study was a preliminary study but obviously the results obtained are potentially significant for the fruit industry. It is important that further work is funded to develop methods to manage and control the fungal rot so that future action can be planned.

Action points for growers

- Apple varieties susceptible to core rots such as Bramley and Cameo should be treated with Bellis (pyraclostrobin + boscalid) or Switch (cyprodonil + fludioxonil) during blossom and petal fall to minimise the risk of *Fusarium* rots.

SCIENCE SECTION

Introduction

HDC project TF 184 on mouldy core identified *Fusarium* spp. as the main fungal species responsible for core rots in both Cameo and Bramley. In HDC project TF 193 *Fusarium* species were also shown to be responsible for storage rots in Bramley, causing stalk, cheek and eye rots, particularly in longer term stored Bramley, where they could account for around 30% of the rotting (actual losses due to rots 2-10%). Several species of *Fusarium* were isolated from core rots and post-harvest rots but the actual species were not identified

Fusarium spp. appear to be increasing in incidence as apple rots in other countries in Europe. Recent investigations in Slovenia on wet core rots in apples cvs. Gloster, Jonagold and Fuji identified *F. avenaceum* as the main cause of the rots and showed high levels of mycotoxins present in apples with wet core rot (Sorensen *et al*., 2009). Recent advances in *Fusarium* taxonomy based on molecular techniques mean that identification of *Fusarium* species is relatively straight forward.

The objective of this project was to identify the main *Fusarium* species responsible for core rots and post-harvest rots in apples and the frequency at which they occur, so that future action can be planned.

Materials and methods

Isolates of *Fusarium* were obtained mainly from two sources. Either from fruit rots collected during apple rot surveys in 2010 / 2011, 2011 / 2012 and 2012/ 2013 or from fruit collected from orchards in August 2012.

Rot survey

Seven pack houses were visited weekly from January-March in 2011-2013. Three were located in East Kent and four south of Maidstone. Visits were also made to pack houses in Hereford. Each of these pack houses graded fruit from their own farm and from other farms. So the survey covered fruit from a number of different farms. At each visit at least 100 rotted fruit were removed from the rot bin or collected from the grader, sampling fruit that was being graded at the time of the visit. Rots were identified visually and numbers recorded. Unknown rots, and particularly those suspected as being *Fusarium* sp, were taken back to the laboratory for identification.

Orchard sampling

Orchards of cvs. Cameo and Bramley were visited during August and September and fruit collected from the orchard floor (fallen fruit) that showed symptoms of rot. Orchards sampled are shown in Table 1. In the laboratory the 100 fruit sample of fallen fruit were immediately chopped and checked for core rot. The incidence of core rot in the sample was recorded and isolations made from the rots onto Potato Dextrose Agar (PDA) to identify the fungi responsible.

Table 1. Orchards sampled in 2012

Farm	Orchard	Cultivar
Hazel Street Farm, Horsmonden	Wirework	Bramley
	No 3	Cameo
River Farm, Staplehurst	4 Row	Cameo
	4 Row	Bramley
Hares Farm, Shottenden		Cameo
Turnover Farm, Wisbech	Pack House	Bramley
Howfield Farm, Chartham	Track	Cameo
	Lower Hambrooks	Bramley
Boxford Farms, Suffolk		Bramley

Isolation of rots

Rotted fruit was washed in running water, surface sterilized in 5% sodium hypochlorite, rinsed in sterile water and blotted dry. The rot leading edge was then plated onto PDA and incubated at 20^{o}C for 7-14 days. *Fusarium* isolates were subbed onto fresh PDA and once free of contamination were sealed and stored at 4^{o}C until required.

Identification of **Fusarium** *species*

In total 120 isolates collected from the sources detailed above were processed. DNA was extracted from the fungal mycelium using the extraction protocol described in Cenis *et al*. (1992). Two genes were partially amplified, internal transcribed spacer (ITS) region and fungal elongation factor 1α (EF1α) using standard polymerase chain reaction (PCR) protocols. Primer sequences and annealing temperatures are shown in Table 2.

Successfully amplified products were purified using Exosap and sent for sequencing using the GATC light run sequencing service (GATC Biotech, Germany).

Both forward and reverse reads were sequenced for the ITS gene whilst only the forward read was sequenced for the EF1α. Sequence data were quality checked and trimmed using Genious 6.0.5 sequencing software. The resulting sequences were queried using the basic local alignment search tool (BLAST) function provided by the FUSARIUM ID database (www.isolate.fusariumdb.org) which hosts 5,535 *Fusarium* sequences from 1,847 isolates representing 76 species. This community-supported database hosts the most comprehensive and up to date sequences from the genus of *Fusarium*, many of the sequences are not yet deposited on the more generic bioinformatics databases such as NCBI. The top match and percentage identity were recorded for each isolate.

Table 2. Primers used for amplification and sequencing

Primer Name	Sequence (5'-3')	Annealing temperature (°C)	References
ITS1	TCCGTAGGTGAACCTGCGG	60	White, 1990
ITS4	TCCTCCGCTTATTGATATGC	60	White, 1990
EF1-983F	GCYCCYGGHCAYGGTGAYTTYAT[1]	60	Rehner, 2001
EF1-1953R	CCRGCRACRGTRTGTCTCAT	60	Rehner, 2001

[1] EF1α primers use degenerate bases, Y=C and T; R= A and G; H= A, C and T.

Results

Identification of Fusarium *species*

The samples collected represent isolates derived from a range of cultivars (mostly Bramley and Cameo), orchard locations and material collected from both orchard and store (Table 3). This representation should capture the *Fusarium* species responsible for mouldy core in UK apples.

PCR amplification and sequencing failures for the ITS gene accounted for the loss of 41 samples during processing meaning that usable sequence data were available for 79 isolates. Of the 79 ITS sequences queried against the *Fusarium* ID database the vast majority (75 samples, 95%) were identified as *Fusarium tricinctum* species complex 1-a. All

but one sequence had percentage identity over 99% which means that we can be confident in the species identity. Only 160bp of sequence data was available for sample R183/12/1, which explains the reduced percentage identity of 96.25%.
A single isolate was identified as *F. incarnatum-equiseti* species complex with a percentage identity of 99.19%, however this identification was based on only 69bp of sequencing data and as such we cannot be confident in this identification. Of the 79 ITS sequences analysed three (4%) had low percentage identities (<90%) when queried against the FUSARIUM ID database. When queried against the NCBI database, which represents all organisms and not just restricted to the *Fusarium* genus, these sequences were identified as *Neonectria galligena* with a percentage identity of 100%.

A second gene (Elongation factor 1α) was partially sequenced to increase the confidence in the species identity and to try to increase the species resolution to within the species complexes. PCR amplification and sequencing failures for the EF1α gene occurred in 46 samples meaning that usable sequence data was available for 74 isolates. Of the 120 isolates processed, sequence data for both genes was available for 54 isolates. A publicly available database, *Fusarium* MLST (http://www.cbs.knaw.nl/Fusarium), enables polyphasic identification using sequences from multiple genes.

The EF1α sequence data collated from the *Fusarium* isolates in this study was amplified from the 3' end of the gene. However the sequence data available for the EF1α gene on the database has a 5' bias, therefore the top matches had very low percentage identities and represented the few species for which sequence data of the whole gene is available. As a result of this data limitation the EF1α sequence data collated in this project will not be useful for species identification until more sequence data is made available.

Alignment of the 74 EF1α sequences from the isolates collected in this study does show that they are all identical which is suggestive that they are within the same species. None of the isolates identified as *Neonectria galligena* using ITS sequence were amplified using EF1α primers suggesting that primer specificity was insufficient to amplify this genus.

Table 3. Molecular identification of isolates in the *Fusarium* collection

Isolate number	Provenance	Variety	S/O[1]	ITS Sequence		EF1a Sequence	Top match on FUSARIUM ID Blast		
							ITS		EF1a
				Fwd	Rev	Fwd[3]	Species	% Identity	
PC14/12	?	?	?	441	x[2]	805	*F. tricinctum* species complex 1-a	99.77%	ND[4]
PC14/12	?	?	?	497	x	539	*F. tricinctum* species complex 1-a	99.79%	ND
R34/12/3/1	FWM	Braeburn	S	x	254	450	*F. tricinctum* species complex 1-a	99.33%	ND
R34/12/3/2	FWM	Braeburn	S	x	x	x	ND		ND
R50/12/1	Breach	Bramley	S	497	191	x	*F. tricinctum* species complex 1-a	99.36%	ND
R52/11/1	NFF	Cameo	S	x	x	x	ND		ND
R52/11/1	NFF	Cameo	S	x	x	x	ND		ND
R52/11/2	NFF	Cameo	S	347	x	101	*F. tricinctum* species complex 1-a	100.00%	ND
R52/11/2	NFF	Cameo	S	x	x	777	ND		ND
R63/12/b/1	NFF	E. Russet	S	216	x	x	*F. tricinctum* species complex 1-a	99.02%	ND
R63/12/b/2	NFF	E. Russet	S	x	x	x	ND		ND
R97/12/a	Red Bank, Herefordshire	Bramley	S	x	501	x	*F. tricinctum* species complex 1-a	99.59%	ND
R97/12/b	Red Bank, Herefordshire	Bramley	S	x	439	659	*F. tricinctum* species complex 1-a	99.77%	ND
R99/11	FWM	Bramley	S	442	x	x	*F. tricinctum* species complex 1-a	99.77%	ND
R99/11	FWM	Bramley	S	x	x	x	ND		ND
R99/11	FWM	Bramley	S	x	x	x	ND		ND
R106/12/a	Breach	Bramley	S	507	503	465	*F. tricinctum* species complex 1-a	99.59%	ND
R106/12/b	Breach	Bramley	S	493	452	x	*F. tricinctum* species complex 1-a	99.42%	ND
R119/12/2/a	Clockhouse Farm	Bramley	S	501	506	546	*F. tricinctum* species complex 1-a	99.58%	ND
R119/12/2/b	Clockhouse Farm	Bramley	S	x	x	153	ND		ND
R119/12/3/a	Clockhouse Farm	Bramley	S	503	498	89	*F. tricinctum* species complex 1-a	99.38%	ND
R119/12/3/b	Clockhouse Farm	Bramley	S	x	x	684	ND		ND
R119/12/4/a	Clockhouse Farm	Bramley	S	488	502	746	*F. tricinctum* species complex 1-a	99.79%	ND
R119/12/4/b	Clockhouse Farm	Bramley	S	430	502	693	*F. tricinctum* species complex 1-a	99.79%	ND
R138/12/3	Wye Fruit	Cox	S	x	x	767	ND		ND
R138/12/4	Wye Fruit	Cox	S	x	x	x	ND		ND
R141/11	?	Bramley	S	x	x	652	ND		ND
R142/12/10	Wye Fruit	Cox	S	310	474	x	*Neonectria galligena*	100.00%	ND
R143/12/2	Wye Fruit	Cox	S	x	x	x	ND		ND
R144/12/1	Breach	Bramley	S	405	410	x	*F. tricinctum* species complex 1-a	99.78%	ND
R144/12/2/a	Breach	Bramley	S	481	506	x	*F. tricinctum* species complex 1-a	99.79%	ND
R144/12/6/b	Breach	Bramley	S	498	503	x	*F. tricinctum* species complex 1-a	99.58%	ND
R170/11	HDC Bramley Trial	Bramley	O	x	502	x	*F. tricinctum* species complex 1-a	99.59%	ND
R170/11	HDC Bramley Trial	Bramley	O	367	457	x	*F. tricinctum* species complex 1-a	99.77%	ND

Table 3. continued

Isolate number	Provenance	Variety	S/O	ITS Sequence		EF1a Sequence	Top match on FUSARIUM ID Blast		
							ITS		EF1a
				Fwd	Rev	Fwd	Species	% Identity	
R173/11	HDC Bramley Trial	Bramley	O	x	x	x	ND		ND
R173/11	HDC Bramley Trial	Bramley	O	x	x	x	ND		ND
R176/11/2	HDC Bramley Trial	Bramley	O	x	x	409	ND		ND
R176/11/2	HDC Bramley Trial	Bramley	O	x	x	x	ND		ND
R176/11/3	HDC Bramley Trial	Bramley	O	497	493	553	*F. tricinctum* species complex 1-a	99.79%	ND
R176/11/3	HDC Bramley Trial	Bramley	O	447	x	599	*F. tricinctum* species complex 1-a	99.77%	ND
R182/12/2	Newling, Turnover Farm, Cambs	Bramley	O	x	x	615	ND		ND
R183/12/1	Newling, Turnover Farm, Cambs	Bramley	O	160	x	803	*F. tricinctum* species complex 1-a	96.25%	ND
R183/12/2	Newling, Turnover Farm, Cambs	Bramley	O	496	501	665	*F. tricinctum* species complex 1-a	99.59%	ND
R183/12/3	Newling, Turnover Farm, Cambs	Bramley	O	x	x	754	ND		ND
R183/12/4	Newling, Turnover Farm, Cambs	Bramley	O	x	x	686	ND		ND
R183/12/5	Newling, Turnover Farm, Cambs	Bramley	O	x	x	521	ND		ND
R183/12/6	Newling, Turnover Farm, Cambs	Bramley	O	421	394	656	*F. tricinctum* species complex 1-a	99.57%	ND
R183/12/7	Newling, Turnover Farm, Cambs	Bramley	O	x	x	x	ND		ND
R183/12/8	Newling, Turnover Farm, Cambs	Bramley	O	320	506	467	*F. tricinctum* species complex 1-a	99.56%	ND
R183/12/10	Newling, Turnover Farm, Cambs	Bramley	O	452	497	666	*F. tricinctum* species complex 1-a	99.58%	ND
R183/12/11	Newling, Turnover Farm, Cambs	Bramley	O	441	479	716	*F. tricinctum* species complex 1-a	99.79%	ND
R183/12/12	Newling, TurnoverFfarm, Cambs	Bramley	O	446	x	556	*F. tricinctum* species complex 1-a	99.77%	ND
R191/12/1	Hazel Street Farm, Horsmonden, Kent	Bramley	O	318	x	655	*F. tricinctum* species complex 1-a	99.37%	ND
R191/12/2	Hazel Street Farm, Horsmonden, Kent	Bramley	O	408	353	798	*F. tricinctum* species complex 1-a	99.50%	ND
R191/12/3	Hazel Street Farm, Horsmonden, Kent	Bramley	O	160	489	639	*F. tricinctum* species complex 1-a	100.00%	ND
R191/12/4	Hazel Street Farm, Horsmonden, Kent	Bramley	O	x	x	303	ND		ND
R191/12/5	Hazel Street Farm, Horsmonden, Kent	Bramley	O	x	x	x	ND		ND
R191/12/6	Hazel Street Farm, Horsmonden, Kent	Bramley	O	271	43	192	*F. tricinctum* species complex 1-a	100.00%	ND
R191/12/7	Hazel Street Farm, Horsmonden, Kent	Bramley	O	x	x	235	ND		ND
R192/12/3	Hazel Street Farm, Horsmonden, Kent	Cameo	O	459	433	x	*F. tricinctum* species complex 1-a	99.78%	ND
R192/12/4	Hazel Street Farm, Horsmonden, Kent	Cameo	O	x	x	x	ND		ND
R192/12/5	Hazel Street Farm, Horsmonden, Kent	Cameo	O	410	x	117	*F. tricinctum* species complex 1-a	99.51%	ND
R192/12/6	Hazel Street Farm, Horsmonden, Kent	Cameo	O	281	x	518	*F. tricinctum* species complex 1-a	99.64%	ND
R192/12/7	Hazel Street Farm, Horsmonden, Kent	Cameo	O	328	421	645	*F. tricinctum* species complex 1-a	99.76%	ND
R192/12/8	Hazel Street Farm, Horsmonden, Kent	Cameo	O	505	495	718	*F. tricinctum* species complex 1-a	99.59%	ND
R194/12/3	Howfield Farm, Canterbury, Kent	Bramley	O	x	x	x	ND		ND
R194/12/8	Howfield Farm, Canterbury, Kent	Bramley	O	x	x	x	ND		ND
R194/12/12	Howfield Farm, Canterbury, Kent	Bramley	O	x	x	328	ND		ND

Table 3. continued

Isolate number	Provenance	Variety	S/O	ITS Sequence		EF1a Sequence	Top match on FUSARIUM ID Blast		
							ITS		EF1a
				Fwd	Rev	Fwd	Species	% Identity	
R194/12/15	Howfield Farm, Canterbury, Kent	Bramley	O	294	319	593	*F. tricinctum* species complex 1-a	99.70%	ND
R194/12/16	Howfield Farm, Canterbury, Kent	Bramley	O	297	370	641	*F. tricinctum* species complex 1-a	99.70%	ND
R195/12/1	Howfield Farm, Canterbury, Kent	Cameo	O	321	414	x	*F. tricinctum* species complex 1-a	99.75%	ND
R195/12/2	Howfield Farm, Canterbury, Kent	Cameo	O	250	190	x	*F. tricinctum* species complex 1-a	99.58%	ND
R195/12/3	Howfield Farm, Canterbury, Kent	Cameo	O	478	x	153	*F. tricinctum* species complex 1-a	99.78%	ND
R195/12/4	Howfield Farm, Canterbury, Kent	Cameo	O	x	x	x	ND		ND
R195/12/5	Howfield Farm, Canterbury, Kent	Cameo	O	217	118	671	*F. tricinctum* species complex 1-a	100.00%	ND
R195/12/6	Howfield Farm, Canterbury, Kent	Cameo	O	286	263	703	*F. tricinctum* species complex 1-a	99.68%	ND
R195/12/7	Howfield Farm, Canterbury, Kent	Cameo	O	308	449	689	*F. tricinctum* species complex 1-a	99.75%	ND
R195/12/8	Howfield Farm, Canterbury, Kent	Cameo	O	496	496	606	*F. tricinctum* species complex 1-a	99.79%	ND
R195/12/9	Howfield Farm, Canterbury, Kent	Cameo	O	x	x	750	ND		ND
R195/12/10a	Howfield Farm, Canterbury, Kent	Cameo	O	x	x	289	ND		ND
R195/12/10b	Howfield Farm, Canterbury, Kent	Cameo	O	x	x	658	ND		ND
R195/12/11a	Howfield Farm, Canterbury, Kent	Cameo	O	289	450	578	*F. tricinctum* species complex 1-a	99.53%	ND
R195/12/11b	Howfield Farm, Canterbury, Kent	Cameo	O	289	433	x	*F. tricinctum* species complex 1-a	99.76%	ND
R195/12/12	Howfield Farm, Canterbury, Kent	Cameo	O	406	x	x	*F. tricinctum* species complex 1-a	99.75%	ND
R195/12/13	Howfield Farm, Canterbury, Kent	Cameo	O	253	216	792	*F. tricinctum* species complex 1-a	99.73%	ND
R195/12/14	Howfield Farm, Canterbury, Kent	Cameo	O	403	x	439	*F. tricinctum* species complex 1-a	99.75%	ND
R195/12/15	Howfield Farm, Canterbury, Kent	Cameo	O	413	423	698	*F. tricinctum* species complex 1-a	99.79%	ND
R195/12/16	Howfield Farm, Canterbury, Kent	Cameo	O	362	493	458	*F. tricinctum* species complex 1-a	99.78%	ND
R195/12/17	Howfield Farm, Canterbury, Kent	Cameo	O	392	463	665	*F. tricinctum* species complex 1-a	99.78%	ND
R195/12/18	Howfield Farm, Canterbury, Kent	Cameo	O	399	438	662	*F. tricinctum* species complex 1-a	99.57%	ND
R195/12/19	Howfield Farm, Canterbury, Kent	Cameo	O	495	462	677	*F. tricinctum* species complex 1-a	99.79%	ND
R195/12/20	Howfield Farm, Canterbury, Kent	Cameo	O	184	147	750	*F. tricinctum* species complex 1-a	100.00%	ND
R196/12/3	Boxford Farm, Suffolk	Bramley	O	117	x	x	*F. tricinctum* species complex 1-a	99.50%	ND
R196/12/5	Boxford Farm, Suffolk	Bramley	O	x	x	x	ND		ND
R196/12/13	Boxford Farm, Suffolk	Bramley	O	326	346	x	*F. tricinctum* species complex 1-a	99.42%	ND
R196/12/19	Boxford Farm, Suffolk	Bramley	O	261	348	x	*F. tricinctum* species complex 1-a	99.71%	ND
R 207/12/2	River Farm, Staplehurst, Kent	Cameo	O	302	301	450	*F. tricinctum* species complex 1-a	99.05%	ND
R 207/12/6	River Farm, Staplehurst, Kent	Cameo	O	314	403	x	*F. tricinctum* species complex 1-a	99.47%	ND
R 207/12/11	River Farm, Staplehurst, Kent	Cameo	O	x	x	x	ND		ND
R 207/12/13	River Farm, Staplehurst, Kent	Cameo	O	227	389	513	*F. tricinctum* species complex 1-a	99.45%	ND
R 207/12/15	River Farm, Staplehurst, Kent	Cameo	O	332	412	683	*F. tricinctum* species complex 1-a	99.51%	ND
R 207/12/16	River Farm, Staplehurst, Kent	Cameo	O	x	x	x	ND		ND

Table 3. continued

Isolate number	Provenance	Variety	S/O	ITS Sequence		EF1a Sequence	Top match on FUSARIUM ID Blast		
							ITS		EF1a
				Fwd	Rev	Fwd	Species	% Identity	
R208/12/6	River Farm, Staplehurst, Kent	Bramley	O	344	439	703	*F. tricinctum* species complex 1-a	99.76%	ND
R208/12/9	River Farm, Staplehurst, Kent	Bramley	O	276	x	663	*F. tricinctum* species complex 1-a	99.27%	ND
R208/12/11	River Farm, Staplehurst, Kent	Bramley	O	x	x	345	ND		ND
R208/12/14	River Farm, Staplehurst, Kent	Bramley	O	x	x	657	ND		ND
R208/12/16	River Farm, Staplehurst, Kent	Bramley	O	422	419	x	*F. tricinctum* species complex 1-a	99.59%	ND
R208/12/19	River Farm, Staplehurst, Kent	Bramley	O	213	300	x	*F. tricinctum* species complex 1-a	99.67%	ND
R209/12/1	Hares Farm, Shottenden, Kent	Cameo	O	229	264	708	*F. tricinctum* species complex 1-a	99.65%	ND
R209/12/2	Hares Farm, Shottenden, Kent	Cameo	O	247	414	x	*Neonectria galligena*	100.00%	ND
R209/12/3	Hares Farm, Shottenden, Kent	Cameo	O	220	452	787	*F. tricinctum* species complex 1-a	100.00%	ND
R209/12/4	Hares Farm, Shottenden, Kent	Cameo	O	342	406	730	*F. tricinctum* species complex 1-a	99.75%	ND
R209/12/6	Hares Farm, Shottenden, Kent	Cameo	O	274	327	x	*F. tricinctum* species complex 1-a	99.69%	ND
R209/12/7	Hares Farm, Shottenden, Kent	Cameo	O	69	x	631	*F. incarnatum-equiseti* species complex	99.19%	ND
R209/12/9	Hares Farm, Shottenden, Kent	Cameo	O	327	383	793	*F. tricinctum* species complex 1-a	99.47%	ND
R209/12/10	Hares Farm, Shottenden, Kent	Cameo	O	373	487	x	*Neonectria galligena*	100.00%	ND
R209/12/11	Hares Farm, Shottenden, Kent	Cameo	O	x	x	517	ND		ND
R209/12/12	Hares Farm, Shottenden, Kent	Cameo	O	x	x	852	ND		ND
R209/12/13	Hares Farm, Shottenden, Kent	Cameo	O	x	x	x	ND		ND
R209/12/18	Hares Farm, Shottenden, Kent	Cameo	O	161	244	753	*F. tricinctum* species complex 1-a	99.65%	ND

[1] origin of isolate either from orchards or stored apples [2] x = no sequence data either due to failed PCR reaction or sequencing reaction [3] only fwd reads were made for EF1α locus [4] ND means no data from Blast search due to 5' bias of EF1α sequence in the FUSARIUM ID database (expanded further in main text).

Discussion

The traditional means to distinguish the *Fusarium* spp. is to differentiate using morphological characteristics. However differences are often discrete, subjective and time consuming and require an in depth knowledge of the genus (Leslie and Summerell, 2006). An alternative approach used increasingly in fungal taxonomy is to use molecular techniques to determine species.

The current project has utilized molecular techniques for the identification of a collected library of *Fusarium* isolates from UK orchards and stores. It is hoped that this technique can be utilized in future projects requiring fungal identification to the species level. The *Fusarium* research community have fully embraced molecular taxonomy and as a result sequence data of many different genes (β-tubulin, intergenic spacer, internal transcribed spacer, rDNA, RNA polymerase subunit 1 and 2 and Translation elongation factor 1α) from 79 species within the genus are available, however this technique is still data limited as experienced in this project with the 5' bias of the EF1α gene sequence. The absence of useful gene sequence data from a second gene meant that the polyphasic identification, whereby two or more sequences are used to determine species identification, could not be utilised.

The power of molecular identification to the genus level is demonstrated by the differentiation of the *Neonectria galligena* from the *Fusarium* species on ITS sequence alone, but in order to get down to species level it is preferable to have sequence data from more than one gene, particularly with species which have low to fair representation which include *F. avenaceum, F.acuminatum and F. tricinctum*. The species identities should therefore be classified cautiously (Geiser *et al*., 2004). Despite this, the high percentage identity between the query sequences and the *F. tricinctum* species complex 1-a sequence means that this finding can be viewed with some confidence. Further sequence data would be required to determine the exact causative species responsible within the species complex which includes *F. acuminatum* and *F. flocciferum*.

Surveys identifying the *Fusarium* species responsible for rotting in apples have been carried out in other countries. In Slovenia *F. avenaceum* was identified as the only species isolated from apples affected with *Fusarium* rot found in Cv. Gloster, Golden Delicious, Fuji and Jonagold orchards (Schroers *et al*., 2008). In Greece a survey of *Fusarium* rots in stored apples (Red Delicious, Golden Delicious, Granny Smith, and Fuji) again showed *F. avenaceum* as the dominant species with some cases of *F. proliferatum*.

Whilst a recent survey of *Fusarium* species isolated from stored apple fruit (cultivars Idared, Jonagold, Golden Delicious and Pink Lady) in Croatia revealed *F. avenaceum* as the dominant species but besides this *F. crookwellense, F. semitectum, F. compactum* and *F. pseudograminearum* were also identified. In the UK *F. lateritium* has been recorded causing a fruit rot on apples (Wormald, 1939; Snowdon,1990) and a bud rot on apple cv. Bramley (Wormald, 1939).

In this current study all of the isolates collected were identified as members of the *F. tricinctum* species complex 1-a. In corroboration of these findings *F. tricinctum* species complex 1-a is considered phylogenetically (Tan and Niessen, 2003) and toxicologically (Desjardins, 2006) similar to *F. avenaceum*.

As part of the sampling strategy isolates were collected from different apple cultivars (particularly Bramley and Cameo which are known to be most susceptible to mouldy core) and from different geographical locations. This sampling strategy was expected to capture the diversity of *Fusarium* species causing mouldy core.

It was expected that isolates collected from a predominantly cereal growing region (such as Turnover Farm, Cambridgeshire) may be different to isolates collected from predominantly apple growing regions (such as those in East Kent). However, all isolates collected were identified as *F. tricinctum* species complex 1-a regardless of where they were collected from. *F. tricinctum* is reported in the literature as having a wide host range including cereals (Castañares *et al*., 2011), onion (Carrieri *et al*., 2013) and apple (Gao *et al.,* 2013) and thus is ubiquitous in many growing regions.

Conclusions

- The species of *Fusarium* responsible for mouldy core in UK apples sampled in this project is *F. tricinctum* species complex 1a.

Knowledge and Technology Transfer

None to date.

Glossary

5’ and 3’ - (5 prime end and 3 prime end) of a nucleic acid sense strand. The terms can be used to describe the opposite ends of a gene. Translation of the sense strand runs from 5’ (‘left’) to 3’ (‘right’).

PCR - (for polymerase chain reaction), a technique for amplifying specific regions of DNA using sequence specific primers and multiple cycles of DNA synthesis

Gene - a region of DNA that controls a discrete hereditary characteristic.

ITS - (for internal transcribed spacer) refers to a piece of non-functional RNA situated between structural ribosomal RNAs on a common precursor transcript. The ITS gene is one of the most widely sequenced regions of fungi. The region contains species specific differences in sequence making it a taxonomically informative region.

EF1 α - (for Elongation factor 1α) refers to a gene encoding an essential part of the protein translation machinery. The gene has phylogenetic utility due to species specific differences in sequence making it a taxonomically informative region.

BLAST - (for basic local alignment search tool) is an algorithm for comparing primary biological sequence information such as nucleotides of DNA sequences. A BLAST search enables the comparison of a query sequence with a library or database of sequences, and identifies library sequences that resemble the query sequence above a certain threshold.

Degenerate nucleotides or bases - is a nomenclature system used in molecular biology whereby in situations where the four nucleotide bases (A, T, G and C) are either unknown or not conserved then degenerate bases can be used in their position to indicate the mix of possible bases at that position.

References

Abbas, H. K., Mirocha, C. J. and Shier, W. T. 1992. Identification and biological activity of chlamydosporol from *Fusarium culmorum* HM-8. Mycopathologia. 118, 115–123.

Bushnell, G. W., Li, Y. L., Poulton, G. A. and Pyrones X.1984. Lateropyrone, a new antibiotic from the fungus *Fusarium lateritium* Nees. Can. J. Chem. 62, 2101–2106.

Carr, S. A., Block, E., Costello, C. E., Vesonder, R. F. and Burmeister, H. R. 1985. Structure determination of a new cyclodepsipeptide antibiotic from *Fusaria* fungi. J. Org. Chem. 50, 2854–2858.

Carrieri, R., Raimo, F., Pentangelo, A. and Lahoz, E. 2013. *Fusarium proliferatum* and *Fusarium tricinctum* as causal agents of pink rot of onion bulbs and the effect of soil solarisation combined with compost amendment in controlling their infections in field. Crop Protection. 43, 31–37.

Castañares E., Stenglein S. A., Dinolfo M. I., and Moreno M. V. 2011. *Fusarium tricinctum* Associated with Head Blight on Wheat in Argentina. Plant disease. 95, 4.

Cenis, J. L 1992. Rapid extraction of fungal DNA for PCR amplification Nucl. Acids Res. 20 (9): 2380

Gao, L. L., Zhang, Q., Sun, X. Y., Jiang, L., Zhang, R., Sun, G. Y., Zha, Y. L., and Biggs, A. R. 2013. Etiology of moldy core, core browning, and core rot of Fuji apple in China. Plant Dis. 97, 510-516.

Grove, J. F. and Pople, M. 1980.The insecticidal activity of beauvericin and the enniatin complex. Mycopathologia. 70, 103–105.

Kotik, A. N. and Trufanova, V. A. 1998. Detection of naphtoquinone fusariotoxin aurofusarin in wheat. Mikol. Fitopatol. 32, 58–61.

Medentsev, A. G. and Akimenko, V. K. 1998. Naphthoquinone metabolites of the fungi. Phytochemistry. 47, 935–959.

Ovchinnikov, Y. A., Ivanov, V. T., Evstratov, A. I., Mikhaleva, I. I., Bystrov, V. F., Portnova, S. L., Balashova, T. A., Meshcheryakova, E. N., and Tulchinsky, V. M. 1974. Enniatin ionophores, conformation and ion binding properties. Int. J. Pept. Protein Res. 6, 465–498.

Rehner S. 2001. Primers for Elongation Factor 1-α (EF1-α). http://ocid.NACSE.ORG/research/deephyphae/EF1primer.pdf

Schroers, H. J., Sorensen, J. L., Thrane, U., Nielsen, K. F., Jerjav M., Munda A., and Frank J.. 2008 High incidence of *Fusarium avenaceum* (Ascomycota, Nectriaceae, Gibberella) and moniliformin in apples showing wet core rot symptoms. J Plant Pathol. 90, Suppl 2 326-333.

Sorensen, J. L., Phipps, R. K., Nielsen, K. F., Schroers, H. J., Frank, J. and Thrane, U. (2009). Analysis of *Fusarium avenaceum* metabolites produced during wet apple core rot. Journal of Agricultural Food Chemistry. 57(4): 1632-9

Snowdon, A. L., 1990. A colour atlas of post-harvest diseases and disorders of fruit and vegetables, Vol. 1 General introduction and fruit, Page 201. Wolfe Scientific

Tan, M. K., and Niessen, L. M., 2003. Analysis of rDNA ITS sequences to determine genetic relationship among, and provide a basis for simplified diagnosis of, Fusarium species causing crown rot and head blight of cereals. Mycological Research. 107: 811-821.

Uhlig, S., Jestoi, M., Knutsen, A. K. and Heier, B. T. (2006) Multiple regression analysis as a tool for the identification of relations between semi-quantitative LC-MS data and cytotoxicity of extracts of the fungus *Fusarium avenaceum* (syn. *F. arthrosporioides*). Toxicon. 48, 567–579.

White, T. J., Bruns, T. D., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ eds. PCR protocols, a guide to methods and applications. San Diego, California: Academic Press. 315-322.

Wormald. 1939. Diseases of fruit and hops. Gen. Editor H C Long. London, Crosby, Lockwood and Sons. 112

