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TF 166e

Influence of agrochemical use and orchard factors on the development of diffuse browning disorder in Cox's Orange Pippin apples

Headline

A link has been established between the application of sprays containing triazole chemicals and the development of diffuse browning disorder in stored Cox apples.

Background and expected deliverables

A hitherto un-described storage disorder (diffuse browning disorder or DBD) of Cox's Orange Pippin apples has resulted in commercial losses of stored fruit since 2000 and was particularly problematic in the 2000-01 and 2004-05 storage seasons. In the initial stages browning of the flesh is localised predominantly towards the calyx end of the fruit but progresses around the fruit and may affect the inner cortex. The disorder is first seen in commercial fruit removed from CA storage in early November and then often progresses rapidly in fruit removed from store and in the worst cases the fruit becomes unmarketable. DBD is regarded as a major threat to the UK top fruit industry and an investigation into the cause of the problem has become a priority.

In 2002, the HDC funded a three-year project (TF 139) to investigate the cause of DBD and although much useful information was gained on factors that influenced the development of the disorder during storage the predisposing factors were not identified (Johnson, 2005, 2006a and 2006b).

HDC funded a further project in 2005 (TF 166d) to examine the possible relationship between DBD and the application and timing of specific types of agrochemicals. Targeting the bloom period to compare different fungicide (and growth regulator) sprays in 2005 didn't produce consistent effects. At many sites there was insufficient DBD to make comparisons of different spray treatments (Johnson, 2006c).

It is clear that a more systematic approach needs to be taken to assess the potential impact of agrochemicals on DBD susceptibility. Dividing orchards and replicating where possible is preferable to spraying different orchards with different regimes. Identifying bins at picking and through storage and grading could prove particularly helpful in assessing the possible contribution of agrochemicals to DBD development.

DBD is a major threat to the UK fruit industry and has affected the confidence of growers to store Cox for more than short-term. Further work is required to confirm the suspicion that factors in the pesticide spray programme induce stresses in the fruit during its development on the tree that progresses to DBD during storage. Growers are being encouraged to leave sectors within orchards that receive nil or minimal pesticide applications and to segregate fruit for storage. In this way the contribution of components of the spray programmes to DBD development can be assessed.

Summary of project and main conclusions

The overall aim of the project was to examine the possible relationship between DBD occurrence and the application and timing of specific types of agrochemicals. More specifically to:

1. Organise the sampling, storage and assessment of fruit from grower trials that compare triazole and non-triazole based spray programmes and other orchard treatments such as thinning and summer pruning.

2. To design and produce a short questionnaire for growers that provides basic information on DBD occurrence and on the permanent features of their Cox orchards and to collate and summarise the responses.

3. To enter in a computer spray data obtained from H.L. Hutchinson Ltd that relates to DBD affected and non-affected orchards. To organise the data and carry out a statistical analysis to identify components of the spray programmes associated with DBD.

Grower spray trials

In 2006, grower trials were carried out to compare triazole and non-triazole based spray programmes. Importantly, these comparisons were carried out **within** orchards on farms considered to be high-risk for DBD.

For the triazole programmeme it was proposed that every spray should include ‘Cultar’ and either ‘Systhane’ or ‘Topas’. It was envisaged that ‘Systhane’ would be applied early-mid season followed by ‘Topas’ sprays mid-late season. Other fungicides (non-triazole) were to be used in addition as required but it was suggested that ‘Regalis’ was omitted from triazole and non-triazole plots in order to avoid over-complicating the trials.

For the non-triazole programme it was proposed that ‘Systhane’, ‘Topas’, ‘Indar’, ‘Rubigan’ (not a triazole but a DMI) and ‘Cultar’ were omitted. A ‘Nimrod’/‘Captan’ programme was suggested for disease control with back-up from ‘Bellis’ (mildew and scab), ‘Karathane’ (Mildew), ‘Stroby’ (scab) and ‘Elvaron Multi’ (scab) as required.

Nine farms were visited by East Malling Research (EMR) staff in late August 2006 to view orchard trials set up by the growers mainly in collaboration with staff from H.L. Hutchinson Ltd. These farms were visited again during the harvesting period and samples of fruit were taken from relevant areas within each of the orchards. Four replicate samples comprised of 50 fruit were taken from each plot. For each sample, one apple was selected at random from each of 50 trees and placed into a sample net and labelled. The procedure was repeated on different rows within each plot. A total of 109 samples was taken during the optimum harvesting period for Cox and transferred to EMR for controlled atmosphere storage. The stores were opened on 12 February 2007 and the sample nets were removed from the bins and after weighing were placed in a room at 20^{o}C for 7 days. Apples seriously affected by fungal decay were discarded and the remaining apples were cut at the calyx end and at the equator and examined for the presence of DBD and other physiological disorders. In addition to samples stored at EMR some of the participating growers labelled bins in their own stores so that an additional assessment could be made on fruit stored commercially. Staff from EMR and Worldwide Fruit Qualytech carried out assessments of the fruit on the growers’ farms and samples were brought back to EMR and examined after a further 7 days at 18 or 20^{o}C.

In six out of nine orchards investigated the inclusion of triazoles in the spray programme resulted in DBD development in the stored fruit (Fig. 1). In the remaining (3) orchards no DBD was found in fruit from trees sprayed with triazole chemicals. DBD was virtually absent in fruit from all orchards where a non-triazole programme was applied. There was clear evidence that the symptoms recognised as DBD in stored Cox apples was affected by the chemical spray programme. It is not known whether the chemical ‘stress’ was induced by individual triazole chemicals or whether the response was due to a combined effect. It is possible (likely) that the time at which the stress occurred was critical but further work is required to confirm this. The inability of triazole sprays to induce DBD in some orchards indicates the involvement of other factors yet to be identified.

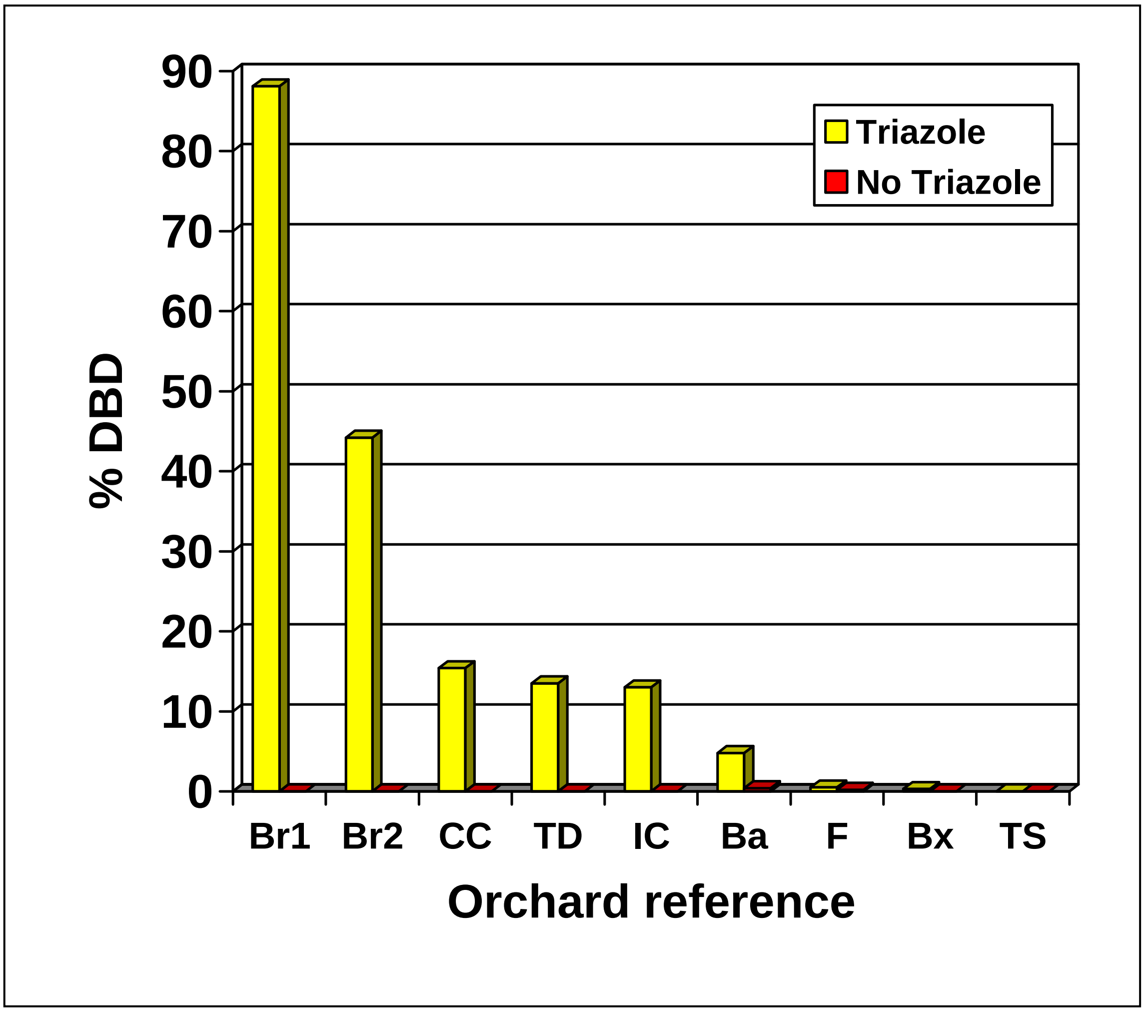

Figure 1. **DBD incidence in fruit (netted samples stored at EMR) sampled from nine commercial orchards where selected rows were sprayed with or without triazole chemicals (2006 crop)**

Grower survey

A survey comprised of 28 questions was prepared by EMR and circulated by HDC to all (levy-paying) apple growers. It was felt that a questionnaire would provide useful information on the extent of the problem within the industry and possible association between DBD occurrence and orchard features and management practices. There were 179 questionnaires completed and returned to EMR via HDC. Of these, 119 were from Cox growers of which 91 stored their fruit. The results of the survey were based on the information supplied by 89 growers that grow and store Cox (2 growers were unable to comment on the DBD susceptibility of their fruit). All the information was subject to statistical analysis.

The main points derived from the survey were as follows:

- In the period 2000-05 inclusive 28% of Cox growers who store fruit had suffered from DBD and 92% of these suffered financial loss
- There was an approximate three-fold increase in occurrence of DBD in 2004 and 2005 compared to the earlier years 2000-2003
- Cox's Orange Pippin and Queen Cox appear to be more affected by DBD than self-fertile Queen Cox and other clones
- There was no evidence of any differences in susceptibility with respect to rootstock
- DBD appears to be more prevalent in fruit from older orchards
- DBD doesn't appear to be related to productivity of the trees
- There is a suggestion that DBD may be more prevalent on lighter soils but there was no association with height of orchards above sea level
- There was no evidence of any effect of thinning or summer pruning on the occurrence of DBD
- There was some evidence that the use of 'Cultar' may be linked to an increased incidence of DBD and limited evidence to suggest use of 'Cultar' at blossom time may have increased incidence of DBD compared to only using 'Cultar' at other times
- There was no evidence of any effect of 'Regalis' use on DBD occurrence
- There did not appear to be any real differences in the incidence of DBD between any of the fungicides normally used and there was no affect of water volumes used for spraying

Analysis of chemical spray data

Ten growers supplied information on agrochemical sprays applied in their orchards via H.L. Hutchinson Ltd. Most of the spray data related to three growing seasons (2002-2004). Not all growers were able to supply information on the type and duration of storage and some had no knowledge of the occurrence of DBD in their fruit.

The data supplied by the growers was collated and the number of each type of fungicide spray (and 'Cultar') was used to correlate with the extent of DBD development. In addition correlation coefficients were calculated for the total number of sprays per month, total sprays applied per season and the total triazole chemicals ('Cultar', 'Systhane' and 'Topas') applied per month and per season.

There were no strong associations between DBD occurrence and the frequency and use of particular types of fungicides or 'Cultar'. A higher degree of correlation between chemical spray programmes and DBD severity may have been expected in view of the clear inducing effects of triazole sprays applied on growers' farms in 2006. However, there are a number of factors that are likely to have contributed to a less clear outcome. Clearly, an accurate assessment of DBD potential is critical in this type of study. In view of the progressive nature of the disorder it is important that the storage period for all consignments reported on is sufficient to allow DBD to develop. For many of the orchards involved in this study the storage period was too short to provide a definite indication of susceptibility. Sixteen percent of the spray data supplied could not used as there was no information available on DBD status of the fruit. In support of the results from the grower spray trials in 2006 the historical data associated the use of the triazole chemicals 'Topas' and 'Cultar' with DBD severity. However there was no correlation between DBD and 'Systhane' the most frequently used triazole fungicide. Surprisingly the best individual relationship was for the non-triazole 'Stroby' applied in May. Generally the results suggest June and to a lesser extent May as critical months for the development of chemical stress in the fruit. Analysis of the historical data was restricted to numbers of sprays applied per month or per season. No account was taken of the rates of application or of combinations of sprays. In view of the reservations about the data set as a whole further analysis seemed unwarranted.

Financial benefits

Growers with orchards that are known to be at risk are restricted to storing fruit short-term. In some years this may result in significant financial loss due to the necessity to market at a time when the markets are traditionally over-supplied with dessert apples. More importantly there is a lack of confidence in storing Cox due to the threat of DBD even where problems have not arisen in the past. It is difficult to quantify the financial implications of forced changes in the marketing strategy for UK Cox. It is easier to cost the loss of consignments of fruit rejected due to the presence of DBD. There are cases of complete losses of stores where retail value of 100 tonnes is in the region of £100,000. The work done previously in HDC project TF139 was helpful in providing advice on how to manage crops of fruit from orchards with a history of DBD. In view of the results obtained from grower trials in 2006 the interim advice to growers with orchards with a history of DBD would be to limit the use of triazole chemicals in their spray programmes particularly where medium to long-term storage is anticipated. Reducing the risk of DBD by modifying the agrochemical spray programme for Cox apples reduces the possibility of crop loss, enables fruit to be stored to its full potential and thereby confers a direct financial benefit of the work.

Action points for growers

- Growers should discuss with their advisers the potential impact of agrochemicals on DBD susceptibility for their particular circumstances
- Non-triazole fungicides should be used in preference to triazole-based fungicides and the use of ‘Cultar’ should be limited in orchards where there is a history of DBD or where medium to long term storage is required
- Where disease control is a priority the most appropriate spray regime may include triazole fungicides. In such cases the duration of storage may be curtailed in order to avoid problems with DBD
- Reduction or avoidance of triazole chemicals in spray programmes should be regarded as an interim measure until further work has been done on the effect of specific chemicals and on the frequency and timing of spray applications

Science Section

Introduction

A hitherto un-described storage disorder (diffuse browning disorder or DBD) of Cox's Orange Pippin apples has resulted in commercial losses of stored fruit since 2000 and was particularly problematic in the 2000-01 and 2004-05 storage seasons. In the initial stages browning of the flesh is localised predominantly towards the calyx end of the fruit but progresses around the fruit and may affect the inner cortex. The disorder is first seen in commercial fruit removed from CA storage in early November and then often progresses rapidly in fruit removed from store and in the worst cases the fruit becomes unmarketable. DBD is regarded as a major threat to the UK top fruit industry and an investigation into the cause of the problem has become a priority.

The HDC funded a three-year project (TF139) to investigate the cause of DBD and although much useful information was gained on factors that may influence the development of the disorder during storage the predisposing factors have not been identified (Johnson, 2005, 2006a and 2006b).

HDC funded a further project in 2005 (TF166) to examine the possible relationship between DBD and the application and timing of specific types of agrochemicals. Targeting the bloom period to compare different fungicide (and growth regulator) sprays in 2005 didn't produce consistent effects. At many sites there was insufficient DBD to make comparisons of different spray treatments (Johnson, 2006c).

It is clear that a more systematic approach needs to be taken to assess the potential impact of agrochemicals on DBD susceptibility. Dividing orchards and replicating where possible is preferable to spraying different orchards with different regimes. Identifying bins at picking and through storage and grading could prove particularly helpful in assessing the possible contribution of agrochemicals to DBD development.

DBD is a major threat to the UK fruit industry and has affected the confidence of growers to store Cox for more than short-term. Further work is required to confirm the suspicion that factors in the pesticide spray programme induce stresses in the fruit during its development on the tree that progresses to DBD during storage. Growers are being encouraged to leave sectors within orchards that receive nil or minimal pesticide applications and to segregate fruit for storage. In this way the contribution of components of the spray programmes to DBD development can be assessed.

Aims of the project

The overall aim of the project was to examine the possible relationship between DBD occurrence and the application and timing of specific types of agrochemicals. More specifically to:

1. Organise the sampling, storage and assessment of fruit from grower trials that compare triazole and non-triazole based spray programmes and other orchard treatments such as thinning and summer pruning.

2. To design and produce a short questionnaire for growers that provides basic information on DBD occurrence and on the permanent features of their Cox orchards and to collate and summarise the responses.

3. To enter in a computer spray data obtained from H.L. Hutchinson Ltd that relates to DBD affected and non-affected orchards. To organise the data and carry out a statistical (multiple regression) analysis of the data to identify components of the spray programmes that are associated with DBD.

Materials and Methods

Grower spray trials

Investigations carried out in 2005 that compared different spray programmes applied in growers' orchards were inconclusive and it was clear that a more systematic approach was needed. Consequently in 2006 further grower trials were carried out to compare triazole and non-triazole based spray programmes. Importantly these comparisons were carried out **within** orchards on farms considered to be high-risk for DBD.

For the triazole programme it was proposed that every spray should include 'Cultar' and either 'Systhane' or 'Topas'. It was envisaged that 'Systhane' would be applied early-mid season followed by 'Topas' sprays mid-late season. Other fungicides (non-triazole) were to be used in addition as required but it was suggested that 'Regalis' was omitted from triazole and non-triazole plots in order to avoid over-complicating the trials.

For the non-triazole programme it was proposed that 'Systhane', 'Topas', 'Indar', 'Rubigan' (not a triazole but a DMI) and 'Cultar' were omitted. A 'Nimrod' / 'Captan' programme was suggested for disease control with back-up from 'Bellis' (mildew and scab), 'Karathane' (mildew), 'Stroby' (scab) and 'Elvaron Multi' (scab) as required.

Actual spray programmes for two of the orchards involved in the 2006 study (ref. CC and IC) are provided in tables 1-4.

Table 1. **Fungicide and growth regulator sprays applied in orchard ref. CC in 2006. Numbers of each type of spray applied are indicated**

Non Triazole	Triazole
Dithianon x3	Dithianon x3
Scala x3	Scala x3
Captan x5	Captan x5
Elvaron Multi x1	Elvaron Multi x1
Systhane x0	Systhane x4
Topas x1	Topas x5
Nimrod x13	Nimrod x5
Cultar x1	Cultar x8

Table 2. **Time of application for triazole chemicals applied in orchard ref. CC in 2006**

Date	Systhane	Topas	Cultar
2 May	✓		
4 May	✓		✓
9 May	✓		
23 May	✓		✓
14 June		✓	✓
21 June		✓	✓
28 June			✓
6 July		✓	✓
19 July		✓	✓
2 August		✓	✓

Table 3. **Fungicide and growth regulator sprays applied in orchard ref. IC in 2006. Numbers of each type of spray applied are indicated**

Non Triazole	Triazole
Dithianon x5	Dithianon x3
Scala x3	Scala x3

Captan x5	Captan x4
Elvaron Multi x0	Elvaron Multi x0
Systhane x0	Systhane x4
Topas x0	Topas x4
Nimrod x9	Nimrod x1
Cultar x0	Cultar x7
Stroby x0	Stroby x3

Table 4. Time of application for triazole chemicals applied in orchard ref. IC in 2006

Date	Systhane	Topas	Cultar
3 May	✓		
15 May	✓		✓
30 May	✓		✓
12 June	✓		✓
26 June		✓	✓
10 July		✓	✓
24 July		✓	✓
2 August		✓	✓

Nine farms were visited by EMR staff in late August 2006 to view orchard trials set up by the growers, mainly in collaboration with staff from H.L. Hutchinson Ltd. These farms were visited again during the harvesting period and samples of fruit were taken from relevant areas within each of the orchards. Four replicate samples comprised of 50 fruit were taken from each plot. For each sample, one apple was selected at random from each of 50 trees and placed into a sample net and labelled. The procedure was repeated on different rows within each plot. A total of 109 samples was taken during the optimum harvesting period for Cox and transported to EMR for storage. Samples were placed into bulk storage bins and loaded into 25-tonne stores. The fruit was cooled to 3.5°C and the stores were sealed. Store oxygen concentration was maintained at 1.2% by automatic ventilation with air (ICA Ltd). Carbon dioxide was maintained at very low concentrations (0.1%) by hydrated lime placed directly in the store with the fruit. The stores were opened on 12 February 2007 and the sample nets were removed from the bins and after weighing were placed in a room at 20°C for 7 days. Apples seriously affected by fungal decay were discarded and the remaining apples were cut at the calyx end and at the equator and examined for the presence of physiological disorders. The incidence of each type of disorder was recorded. In addition to samples stored at EMR some of the participating growers labelled bins in their own stores so that an additional assessment could be made on fruit stored commercially. Staff from EMR and Worldwide Fruit Qualytech carried out assessments of the fruit on the growers' farms and samples were brought back to EMR and examined after a further 7 days at 18 or 20°C.

It was possible to analyse some data using an analysis of variance (ANOVA) with GENSTAT 9 statistical software.

Grower survey

A survey comprised of 28 questions was prepared by EMR and circulated by HDC to all (levy-paying) apple growers. It was felt that a questionnaire would provide useful information on the extent of the problem within the industry and any possible association between DBD occurrence and orchard features and management practices. There were 179 questionnaires completed and returned to EMR via HDC. Of these 119 were from Cox growers of which 91 stored their fruit. The results of the survey were based on the information supplied by 89 growers that grow and store Cox (2 growers were unable to comment on the DBD susceptibility of their fruit). All the information from each grower was entered into an excel spreadsheet and analysed using GENSTAT 9 statistical software. Since most of the responses to the questions were in the form of ‘Yes’ or ‘No’ it was generally appropriate to carry out a Chi-squared (χ^2) test on the data. In some cases it was possible to analyse data using an analysis of variance (ANOVA) with GENSTAT 9 statistical software.

Analysis of chemical spray data

Ten growers supplied information on agrochemical sprays applied in their orchards via H. L. Hutchinson Ltd. Most of the spray data related to three growing seasons (2002-2004). Not all growers were able to supply information on the type and duration of storage and some had no knowledge of the occurrence of DBD in their fruit. A summary of the type of information received additional to spray details is provided in Table 5. The multiple of orchards and years provided 63 data points for analysis. However, it was only possible to analyse data for 53 data points since on 10 occasions there were no details of DBD occurrence provided. The total number of sprays of each type of fungicide and ‘Cultar’ were as follows:

‘Cultar’ 524, ‘Systhane’ 361, ‘Topas’ 294, ‘Captan’ 285, ‘Dithianon’ 211, ‘Stroby’ 207, ‘Nimrod’ 86, ‘Scala’ 68, ‘Elvaron Multi’ 67, ‘Bavistin’ 58, sulphur 21, ‘Karathane’ 4 and others 25

Table 5. **Information provided by growers additional to spray data**

Grower ref.	Orchard ref.	Year	Storage		DBD
			Type	Duration	
Bo	St	2004	No details	No details	Severe*
	Co	2004	No details	No details	Severe*
	Gr	2004	No details	No details	Severe*
	MP	2004	No details	No details	Severe*
Bo	St	2005	No details	No details	None*
	Co	2005	No details	No details	None*
	Gr	2005	No details	No details	None*
	MP	2005	No details	No details	None*
Bar	Pl	2004	CA	February	Severe
	Pl	2005	No details	No details	No details
	Re	2004	No details	No details	No details
	Re	2005	No details	No details	No details
Bal		2005	No details	No details	No details
CD	D	2002	CA	January	Low
		2003	CA	January	Moderate
		2004	CA	January	Moderate
	BS	2002	CA	December	None
		2003	CA	February	None
		2004	CA	December	Severe
MF	Ap	2004	CA	November	None
Fi	CL	2003	No details	No details	No details
		2004	CA	November	None
	Ac	2002	CA	January	None
		2003	CA	February	None-Low
		2004	CA	February	Low-Severe
	Pi	2002	CA	December	None
		2003	CA	December	None
		2004	CA	January	Low-Severe
	Be	2002	CA	December	None
		2003	CA	January	Severe
		2004	CA	November	No details
	Br	2002	CA	January	None
		2003	CA	February	None
		2004	CA	January	None
	Bu	2002	CA	January	Severe
		2003	CA	February	None
		2004	Air + SF	December	None
	CD	2002	CA	January	Severe
		2003	CA	January	Moderate
		2004	CA	November	No details
	Co	2002	Air	October	No details
		2003	Air	November	No details
		2004	Air + SF	January	None-Severe
	Et	2002	CA	January	None
		2003	CA	January	Severe
		2004	Air + SF	December	None
	Ma	2002	CA	January	Low
		2003	CA	February	Low
		2004	CA	January	None-Moderate
	OW	2002	CA	December	None
		2003	CA	February	None
		2004	CA	January	Severe
Fo		2002	CA	No details	None
		2003	CA	No details	None
		2004	CA	No details	Moderate
Th		2003	No details	No details	Low

Grower ref.	Orchard ref.	Year	Storage		DBD
			Type	Duration	
		2004	No details	No details	Severe
		2005	No details	No details	No details
Wh		2002	CA	No details	None
		2003	CA	No details	None
		2004	CA	No details	None
4D	QC	2003	CA	No details	None
		2004	CA	No details	None

*-general situation for the farm as a whole

The data supplied by the growers was collated and the number of each type of spray applied each month from March to August was entered into an Excel spreadsheet prior to analysis using GENSTAT 9 statistical software. Correlation coefficients were calculated for the extent of DBD (none, low, moderate or severe was scored as 0, 1, 2 and 3 respectively) and the number of each type of spray applied in each month. In addition correlation coefficients were calculated for the total number of sprays per month, total sprays applied per season and the total triazole chemicals ('Cultar', 'Systhane' and 'Topas') applied per month and per season. Multiple linear regression analyses were carried out with the numbers of sprays of each chemical applied each month as explanatory variables.

Results and Discussion

Grower spray trials

Fruit samples stored at EMR

In six out of nine orchards investigated, the inclusion of triazoles in the spray programme resulted in DBD development in the stored fruit (Table 6). In the remaining (3) orchards no DBD was found in fruit from trees sprayed with triazole chemicals. DBD was virtually absent in fruit from all orchards where a non-triazole programme was applied. There were no significant effects of spray programme on weight loss, rotting or incidence of other physiological disorders in the fruit.

Table 6. **The effect of including triazole chemicals in agro-chemical spray programmes on the weight loss and incidence (%) of physiological disorders in Cox apples sampled in September 2006 from nine commercial orchards. Fruit was examined after storage in 1.2% O_2 and <1%CO_2 at 3.5°C until 12 February 2007 followed by a further 7 d at 20°C. (SED–Standard Error of the Difference between means, df–degrees of freedom)**

Site Ref.	Triazoles	Wt Loss (%)	Rotting (%)	DBD (%)	Bitter Pit (%)	Calyx Browning (%)	Cortex Browning (%)
BR1	Yes	6.2	7.3	81.1	0	0	2.5
	No	5.6	4.0	0	0	0	0
SED 8df		0.27	4.77	5.56	-	-	3.10
BR2	Yes	6.1	1.0	44.2	0	0	0
	No	5.9	6.0	0	2.1	0	0
SED 2df		0.35	6.08	19.75	2.13	-	-
CC	Yes	5.5	3.9	15.4	6.0	2.0	3.0
	No	5.2	6.0	0	3.1	0	0.5
SED 6df		0.23	2.79	3.03	2.40	1.96	1.80
TD	Yes	4.8	6.5	13.5	8.5	0	10.8
	No	4.5	5.0	0	4.1	0	10.7
SED 6df		0.29	3.55	6.78	4.57	-	6.42
IC	Yes	5.3	3.0	13.0	12.0	0	6.0
	No	5.3	3.0	0	15.2	0	3.1
SED 2df		-	4.24	3.00	3.18	-	3.06
BA	Yes	4.4	3.7	4.8	1.0	0.3	1.7
	No	4.7	3.3	0.4	3.5	0	0
SED 10df		0.30	2.20	0.93	2.42	0.33	0.97
F	Yes	3.4	0.7	0.5	0.4	0	0.4
	No	3.3	0.7	0.2	1.0	0	0.2
SED 19df		0.12	0.52	0.43	0.51	-	0.31
BX	Yes	5.7	3.4	0.3	4.3	2.0	0.3
	No	5.2	1.0	0	2.7	0	0
SED 10df			2.38	0.33	2.00	0.91	0.33
TS	Yes	4.4	4.5	0	11.1	0	12.1
	No	4.2	3.5	0	4.1	0	5.2
SED 6df		0.36	2.80	-	3.22	-	3.31

Samples removed from bins after commercial storage (Tables 7-10)

Four farms were visited to examine fruit from on-farm storage. On three of the farms (orchard references BR1, CC and IC) it was possible to remove fruit directly from the bins. At the remaining site (orchard reference BA) the grower had sampled fruit prior to the visit. With the exception of fruit from orchard reference BA DBD was present only in fruit from trees sprayed with triazole chemicals. Generally, there was insufficient DBD in fruit from two of the orchards (Reference IC and BA) to establish any possible treatment effects. In fruit examined from the other orchards (Reference BR1 and CC) the effect of triazoles in promoting DBD was clear. In general higher incidences of DBD were found in the samples taken from the trees at harvest and stored at EMR. There was a large variation in DBD incidence in 50-fruit samples taken from different rows within the plots sprayed with triazoles. It was therefore to be expected that the samples taken from the surface of the bins did not provide the same incidence of DBD as the 50-fruit samples. The bins were likely to represent a more restricted area of the orchard than the 50-fruit samples.

Table 7. **Storage disorders in Queen Cox apples from an orchard trial (Ref. BR1). For the ex-store assessment 60 apples (20 per bin) from each treatment were examined. For the examination after shelf-life (8d at 18ºC) 150 apples (50 per bin) from each treatment were examined**

	Triazoles	**No Triazoles**
Ex-store (5.3.07)		
Bitter pit (%)	3.3	0
DBD (%)	8.3	0
Ex-8d at 18ºC (13.3.07)		
Bitter pit (%)	0	0
DBD (%)	17.6	0
Calyx end browning (%)	2.0	0

Table 8. **Storage disorders in Queen Cox apples from an orchard trial (Ref. CC). For the ex-store assessment 200 apples (10 per bin) from each treatment were examined. For the examination after shelf-life (7d at 18ºC) 400 apples (20 per bin) from each treatment were examined**

	Triazoles	**No Triazoles**
Ex-store (12.2.07)		
Bitter pit (%)	2.0	0.5
DBD (%)	5.0	0
Ex-7d at 18ºC (19.2.07)		
Bitter pit (%)	9.5	11.8
DBD (%)	9.5	0
Core flush (%)	0.7	0
Low temp. breakdown (%)	0.2	0

Table 9. Storage disorders in Queen Cox apples from an orchard trial (Ref IC). For the ex-store assessment 190 apples (10 per bin) from each treatment were examined. For the examination after shelf-life (7d at 18ºC) 380 apples (20 per bin) from each treatment were examined

	Triazoles	No Triazoles
Ex-store (11.1.07)		
Bitter pit (%)	1.6	3.7
Water core (%)	0	1.6
DBD (%)	0.5	0
Ex-7d at 18ºC (18.1.07)		
Bitter pit (%)	3.7	17.8
Water core (%)	0.3	1.0
DBD (%)	0.5	0
Core flush (%)	0	0.5
Senescent breakdown (%)	0	1.0
Low temp. breakdown (%)	3.9	0

Table 10. Storage disorders in Queen Cox apples from an orchard trial (Ref. BA). Fruits were provided in green supermarket trays (2 per treatment) and examined after shelf-life (8d at 18ºC)

	Triazoles	No Triazoles
Ex-8d at 18ºC (13.3.07)		
Bitter pit (%)	1.0	1.0
DBD (%)	1.5	1.0
Flesh breakdown (%)	5.4	9.5
Calyx end browning (%)	0.5	1.5

Grower survey

Of the questionnaires returned, 89 indicated that they grew and stored Cox and knew the storage history of their fruit, and it is these that have subsequently been analysed. Twenty-five growers (28%) had suffered from DBD. Of the 25 who had suffered DBD, 23 (92%) reported suffering financial loss. Over a six-year period 2000-05 inclusive, there were most reports of problems in 2004 and 2005 with an approximate three-fold increase compared to the earlier years 2000-2003. Clearly, growers have become more familiar with the problem in recent years and are more able to distinguish DBD symptoms from other disorders, such as bitter pit. There needs to be some caution about interpreting these results as an accurate reflection of seasonal risk. The percentage of fruit affecting consignments varied with as many growers reporting more than 20% affected fruit as those reporting less than 5% affected. Only 33% of growers who had suffered from DBD were able to identify susceptible orchards.

As expected, Queen Cox was the most popular clone in production, with three times that of Cox's Orange Pippin, self-fertile Queen Cox and other clones. The proportion of Cox's Orange Pippin and Queen Cox suffering from DBD appears greater than that for self-fertile Queen Cox and other clones (Table 11). Carrying out a χ^2 test for the proportion affected with respect to clone, a statistic of 8.86 on 3 df was obtained, which has a probability p=0.031; thus there is some statistical evidence of a significant difference.

Table 11. Cox clones currently under production as specified by 86 growers and those affected by diffuse browning disorder (DBD). Multiple answers were allowed thus 153 clones specified

Clone	Cox's Orange Pippin	Queen Cox	Self-fertile Queen Cox	Other Clones
Clones grown	25	77	23	28
% of growers with clones	29	90	27	33
Clones affected by DBD	7	19	2	1
% of those grown affected by DBD	28.0	24.7	8.7	3.6

Most of Cox production was on M9 rootstocks (80% of growers) followed by MM106 (49%), M26 (19%) and others (14%). There was no evidence of any differences in susceptibility to DBD with respect to rootstock. There was an indication of DBD being more prevalent in the older orchards (11-20 and >20 years) compared to orchards of <5 and 5-10 years. However, since different respondents gave different numbers of responses it is not legitimate to do a formal test for 'significance'. There appears to be no difference in mean estimated productivity (yield) between affected and unaffected orchards at the DBD sites and no difference in the yield from affected and non-affected sites. The descriptions of soil types provided by the growers (79 respondents) were placed into 6 categories which aligned with the texture groups used by ADAS and the Soil Survey of England and Wales (MAFF, 1984). DBD occurred in orchards on all soil types (Table 12).

Table 12. The soil types for sites affected or unaffected by diffuse browning disorder (DBD)

	1	2	3	4	5	6
	Sands	**Light loams**	**Light silts**	**Medium loams**	**Medium silts**	**Clays**
DBD: No	4	20	7	9	4	13
DBD: Yes	3	5	5	6	1	2
Total no.	7	25	12	15	5	15
% affected by DBD	42.9	20.0	41.7	40.0	20.0	13.3

The numbers in each soil category are really too small for a formal χ^2 test. However, types 1, 3 and 4 do appear to have a greater proportion of DBD than types 2, 5 and 6.

There was a tendency for DBD to be worse on the lighter soils although the lower incidence on light loam sites conflicts with this association. There is no evidence of any effect of thinning or summer pruning on the incidence of DBD. Prior to 2005 67% of growers had used ‘Cultar’. There is some evidence that the use of ‘Cultar’ may be linked to an increased incidence of DBD (Table 13). However, there were growers who had suffered DBD who had not used ‘Cultar’ and conversely growers who had used ‘Cultar’ and not experienced DBD. The average number of ‘Cultar’ applications was 4.3 for those without DBD (30 respondents) compared to 5.5 for those with DBD (20 respondents); carrying out a 1-way ANOVA for this gives a probability level of p=0.066, so borderline in indicating a significant difference (increase of about 1 spray for those with DBD compared to those without). There is limited evidence to suggest use of ‘Cultar’ at blossom time may have increased the incidence of DBD compared to only using ‘Cultar’ at other times. Overall, 48% of growers used ‘Regalis’ in 2005 but there was no evidence of any effect on incidence of DBD.

Table 13. The effect of ‘Cultar’ application on the occurrence of diffuse browning disorder (DBD)

DBD	Cultar		Total	Cultar at blossom time		Total
	No	**Yes**		**No**	**Yes**	
No	23	34	57	26	2	28
Yes	4	21	25	16	5	21
Total	27	55	82	42	7	49
% with DBD	**14.8**	**38.2**		**38.1**	**71.4**	

There was no evidence that water volumes typically used for spraying agrochemicals were associated with DBD occurrence. The mean for those without DBD was 290 l per ha compared to 305 l per ha for those with DBD and the range of responses was wide: 90-1100 l per ha for those without DBD compared to 55-1100 l per ha for those with DBD. Height of orchards above sea level did not relate to DBD occurrence. The mean for those without DBD was 56.9 metres compared to 58.8 metres for those with DBD.

Table 14. Fungicide use by growers affected or unaffected by diffuse browning disorder

Fung\DBD	Fungicides normally used					Fungicides frequently used				
	No	Yes	Total	% use*	% DBD†	No	Yes	Total	% use*	%DBD†
Bavistin	13	4	17	20	24	4	1	5	6	20
Bellis	2	1	3	4	33	0	1	1	1	100
Captan	50	18	68	80	27	39	17	56	66	30
Dithianon	48	21	69	81	30	31	17	48	57	35
Dodine	9	4	13	15	31	2	0	2	2	0
ElvaronM	24	9	33	39	27	7	5	12	14	42
Indar	9	3	12	14	25	3	1	4	5	25
Karathane	9	4	13	15	31	2	0	2	2	0
Mancozeb	2	1	3	4	33	1	1	2	2	50
Nimrod	32	11	43	51	26	7	7	14	17	50
Rubigan	1	1	2	2	50	0	0	0	0	0
Scala	32	18	50	59	36	12	5	17	20	29
Stroby	35	16	53	62	30	8	5	13	15	39
Sulphur	5	4	9	11	44	1	1	2	2	50
Systhane	46	22	68	80	32	34	16	50	59	32
Thiram	2	2	4	5	50	1	0	1	1	0
Topas	32	13	45	53	29	21	8	29	34	28
Others	2	1	3	4	33	1	0	1	1	0

* - number using as a % of total respondents (85)
† - number of those using fungicide with DBD present, as a % of total number for that fungicide

In terms of fungicides normally used, 'Captan', 'Dithianon' and 'Systhane' were the most popular (~80% of respondents used them), with 'Stroby', 'Scala', 'Topas' and 'Nimrod' next (more than 50%) (Table 14). Although there is not sufficient data for formal testing, it does not look as if there are any real differences of incidence of DBD between any of the fungicides normally used (similar percentages throughout, with 'Rubigan', 'Thiram' and sulphur having the highest incidence [45-50%] but not normally used by many people). The fungicides most frequently used were the same – 'Captan', 'Dithianon' and 'Systhane' (~60%), followed by 'Topas' (34%). Numbers are even more sparse here so the incidence of DBD varying from 0 – 100% is not that likely to indicate any real differences, based on so few numbers. It should be emphasised that no information was requested on the frequency or rates of application of any of the fungicides used by growers or on timing of sprays or combinations of chemicals in the sprayer tanks.

Analysis of chemical spray data

Analysis of the spray data derived from selected growers did not indicate strong associations between DBD occurrence and the frequency and use of particular types of fungicides or 'Cultar'. From an analysis of the monthly spray data, the strongest correlations with DBD severity were with ‘Stroby’ applied in May (r=0.498), ‘Topas’ applied in June (r=0.402) and ‘Cultar’ applied in May (r=0.300) and June (r=0.395). These were the only significant correlations from 78 possible. In view of the results from the grower spray trials in 2005 it was reassuring that the use of the triazole chemicals ‘Topas’ and ‘Cultar’ was positively correlated with DBD severity. However there was no correlation between DBD and ‘Systhane’ the most frequently used triazole fungicide. Where data for the total sprays of each chemical per season were analysed only ‘Stroby’ (r=0.403) and ‘Cultar’ (r=0.308) gave significant correlations with DBD. In a regression analysis ‘Stroby’, ‘Topas’ and ‘Cultar’ accounted for 20.6, 17.5 and 10.5% of the variation in DBD severity with all other chemicals accounting for less than 10%. In a multiple regression analysis of DBD severity the best fit (26% variance accounted for) was achieved with June application of triazoles (total number of ‘Systhane’, ‘Topas’ and ‘Cultar’ sprays). Generally the results suggest June and to a lesser extent May as critical months for the development of chemical stress in the fruit.

A higher degree of correlation between chemical spray programmes and DBD severity may have been expected in view of the clear inducing effects of triazole sprays applied on growers’ farms in 2006. However, there are a number of factors that are likely to have contributed to a less clear outcome:

- Ten of the 63 data sets could not be used as there was no information available on the DBD status of the fruit
- Fourteen of the 63 data sets relate to fruit stored pre-Christmas. Since DBD is a progressive disorder that is generally only apparent from December the duration of storage is critical in determining the susceptibility of any consignment to DBD. Consignments free of DBD may have developed the disorder if examined later in the storage period. This would have a major effect on any correlations
- It was not possible to restrict data to fruit stored for minimum periods as no storage duration information was indicated for 24 of the 63 data sets
- The reliability of the DBD data can be questioned further and much depends on the method of sampling used to form samples for monitoring purposes. There was clearly variation in DBD in samples at some sites and in the worst case DBD score ranged from none to severe
- The categories for DBD severity are too few to expect strong correlations. A larger range in the DBD data, e.g. by using percentage of fruit affected would have increased the potential for stronger correlations

- Although 10 growers provided information the data was skewed with 50% of the data being provided by one of the growers. The survey would have benefited from inputs from a larger number of growers

Analysis was restricted to numbers of sprays. No account was taken of the rates of application or of combinations of sprays. In view of the reservations about the data set as a whole further analysis would seem unwarranted.

Conclusions

In the 2006 trials there was clear evidence that the symptoms that we associate with DBD in stored Cox apples was affected by the chemical spray programme. It is not known whether the chemical 'stress' was induced by individual triazole chemicals or whether the response was due to a combined effect. It is possible (likely) that the time at which the stress occurred was critical but further work is required to confirm this. The inability of triazole sprays to induce DBD in some orchards indicates the involvement of other factors yet to be identified.

The main points derived from the survey were as follows:

- In the period 2000-05 inclusive 28% of Cox growers who store fruit had suffered from DBD and 92% of these suffered financial loss
- There was an approximate three-fold increase in DBD occurrence in 2004 and 2005 compared to the earlier years 2000-2003
- Cox's Orange Pippin and Queen Cox appear to be more affected by DBD than self-fertile Queen Cox and other clones
- There was no evidence of any differences in susceptibility with respect to rootstock
- DBD appears to be more prevalent in fruit from older orchards
- DBD doesn't appear to be related to productivity of the trees
- There is a suggestion that DBD may be more prevalent on lighter soils but there was no association with height of orchards above sea level
- There was no evidence of any effect of thinning or summer pruning on the occurrence of DBD

- There was some evidence that the use of 'Cultar' may be linked to an increased incidence of DBD and limited evidence to suggest that the use of 'Cultar' at blossom time may have increased incidence of DBD compared to only using 'Cultar' at other times
- There was no evidence of any effect of 'Regalis' use on DBD occurrence
- It does not look as if there are any real differences of incidence of DBD between any of the fungicides normally used and there was no affect of water volumes used for spraying

Analysis of historical spray data derived from selected growers did not indicate strong associations between DBD occurrence and the frequency and use of particular types of fungicides or 'Cultar'. A higher degree of correlation between chemical spray programmes and DBD severity may have been expected in view of the clear inducing effects of triazole sprays applied on growers' farms in 2006. However, there are a number of factors that are likely to have contributed to a less clear outcome. Clearly, an accurate assessment of DBD potential is critical in this type of study. In view of the progressive nature of the disorder it is important that the storage period for all consignments reported on is sufficient to allow DBD to develop. For many of the orchards involved in this study, the storage period was too short to provide a definite indication of susceptibility. Sixteen percent of the spray data supplied could not be used as there was no information available on DBD status of the fruit. In support of the results from the grower spray trials in 2005, the historical data associated the use of the triazole chemicals 'Topas' and 'Cultar' with DBD severity. However, there was no correlation between DBD and 'Systhane', the most frequently used triazole fungicide. Analysis of the historical data was restricted to numbers of sprays applied per month or per season. No account was taken of the rates of application or of combinations of sprays. In view of the reservations about the data set as a whole further analysis would seem unwarranted.

Future funding

Defra have agreed to provide funding for two years to enable strategic research into the cause of diffuse browning disorder in Cox apples. The HDC has agreed to add financial support to the Defra project which will involve collaboration with the Institute of Food Research in Norwich.

Technology transfer

Progress report to growers who attended the MFFS/EMRA Storage Day held at EMR on 27 March 2007

Discussion of DBD problems in the 2006 crop and the way forward. Meeting held at EMR on 2 April 2007

HDC News May 2006. Diffuse causes of a diffuse disorder

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