Project title	Development of diffuse browning disorder in relation to physiological and biochemical changes in apple fruit during development and storage
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Project leader:	David Johnson, East Malling Research
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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.

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Grower Summary

Headline

- Diffuse browning disorder (DBD) can be induced by applications of triazole fungicides or paclobutrazol alone but in combination higher levels of DBD can be induced in stored Cox apples.

Background and expected deliverables

The storage disorder diffuse browning disorder (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 making DBD a major threat to the UK top fruit industry.

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. HDC funded a further series of projects (TF 166a-f) to examine the possible causes of DBD and Defra agreed to fund research to identify the underlying cause of diffuse browning disorder, on the understanding that HDC would also support the project. This jointly funded project, TF 166g (Defra project F00308), is reported here.

There was evidence prior to commencement of this project that the development of DBD in stored Cox apples was related to the application of particular compounds used in the spray programme during fruit development (see final report for project TF 166e). It appeared that the inclusion of fungicides and growth regulators classified as triazole compounds resulted in stresses in the fruit which initiated the development of DBD during the storage period. Triazoles are any of several compounds with composition $C_2H_3N_3$, having a 5-membered ring of 2 carbon atoms and 3 nitrogen atoms. Chemicals with this structure can act as fungicides e.g. myclobutanil ('Systhane') and penconazole ('Topas'), plant growth modifiers e.g. paclobutrazol ('Cultar') or herbicides e.g. Amitrole ('Aminotriazole').

This research aims to identify potential internal regulators of DBD susceptibility and to develop hypotheses on the most likely stress factors affecting fruit during their development

on the tree. Changes in the chemical profile of apples during development and storage and in the physiology of fruit at harvest and during storage will be measured and related to the development of DBD. Molecular techniques will be used to study the expression of genes known to be involved in regulating stress in plants. Changes in the anatomical structure in apple flesh that precedes the development of DBD will be observed. The research will offer opportunities to develop and deliver practical methods to avoid or prevent DBD and may indicate possibilities for developing diagnostic tests. The physiological studies of fruit ripening and disorder expression may pave the way for methods of screening commercial consignments of fruit at harvest.

The objectives of the project are as follows:

1. To measure changes in the cellular chemical profile of apples during development and storage and relate these to the development of diffuse browning disorder
2. To measure changes in the physiology of fruit at harvest and during storage and relate these to the development of diffuse browning disorder
3. To characterise the morphology of DBD in affected apples by identifying and defining the sequence of events from early onset to the development and spread of pigmented necrotic cells within the outer cortex tissue
4. Preliminary investigation of stress induced pathways by analysis of gene transcripts
5. To communicate results and facilitate technology transfer

Summary of the project and main conclusions

Experimental treatments 2007

Trials were set-up at EMR and on 7 commercial farms in Kent to investigate further the possible link between DBD development and application of triazole compounds during fruit development. Trial orchards were divided to allow the application of different spray programmes. In all 8 orchards a non-triazole programme (excluded the growth regulator paclobutrazol (‘Cultar’) and the fungicides myclobutanil, penconazole and fenbuconazole) was compared with a programme that included triazole compounds. In 6 of the orchards two additional treatments were applied i.e. a spray programme that included paclobutrazol but no triazole fungicides and a programme that included triazole fungicides but no paclobutrazol.

Samples of fruit were taken from the trial orchards in June, July and August and prepared for analysis. Samples were also taken at commercial harvest for storage under refrigeration and in controlled atmosphere (CA) conditions at EMR. Treatments applied to the fruit prior to storage included application of a chemical antioxidant (diphenylamine), an ethylene action

inhibitor (1-methylcyclopropene) and a delayed cooling treatment (7 days at 20ºC). These post-harvest treatments tested the possibility of delaying or preventing the development of clinical symptoms of DBD in the stored fruit. Samples of fruit were removed from storage during December to measure respiration and ethylene production rates and in February all samples were removed from store and examined internally for the presence of DBD and any other disorders. Samples of fresh apples from CA storage were provided to the Institute of Food Research (IFR) to allow the anatomical investigations to be carried out and samples of tissue were prepared and frozen in readiness for chemical analysis by IFR.

Experimental results 2007

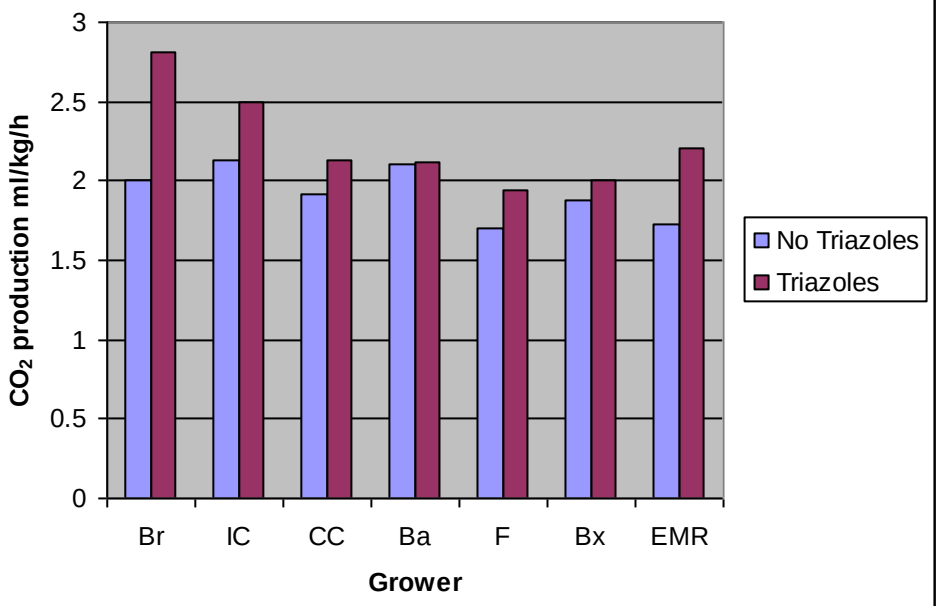
DBD did not develop in fruits from plots that were sprayed with non-triazole compounds (Table 1). DBD occurrence in the non-triazole treatment in orchard reference CC was explained by the fact that triazoles had been applied from late June onwards. DBD developed in fruits from plots sprayed with triazole compounds which confirm previous evidence that the disorder is induced by chemical stresses imparted by sprays applied during fruit development. The incidence of DBD varied markedly between orchards and was not associated with the number or quantity of triazole compounds applied. Clearly fruit from some orchards are more able to withstand stresses imposed by chemical sprays. It was clear from the storage results that DBD could be induced by fungicides or paclobutrazol alone but in combination they induced most DBD. This is the first time that an additive effect of triazole fungicides and a triazole growth regulator has been demonstrated which has major practical implications for growers. The development of clinical symptoms of DBD was not ameliorated by the application of post-harvest treatments.

Table 1. Effect of triazole sprays on the incidence (%) of Diffuse Browning Disorder (DBD) in CA-stored (<1% CO_2 + 1.2% O_2 at 3.5ºC) Cox apples from 8 orchards in 2007

Triazole sprays		**Orchard Reference**							
Fungicide	**Cultar**	**Br**	**CC**	**TD**	**EMR**	**IC**	**Bx**	**F**	**Ba**
No	No	0	9.1	0	1	0	0	0	0
No	Yes	0	-	26.6	-	0	3.2	0	-
Yes	No	56.6	-	0	13.7	21.2	5.0	0	-
Yes	Yes	75.7	35.0	40.4	30.9	18.5	22.0	1.0	0
SED (4 df)		8.75	3.11	4.92	1.48	5.00	2.33	-	-

Physiological studies showed a consistently higher respiration rate in stored fruit treated with triazoles (Fig. 1. This heightened respiration may have existed in fruits since the chemical stresses were exerted during development but it is more likely that this occurred coincident with cellular disorganisation prior to the development of clinical symptoms.

Fig. 1. Effect of triazoles on the respiration rate of Cox apples immediately ex-CA (19.12.07)



In the analytical work being carried out by IFR, nuclear magnetic resonance (NMR) nuclear magnetic resonance (NMR) spectra have been obtained for sets of cores from three different regions of DBD affected apples (from one orchard): diseased tissue; adjacent but apparently unaffected tissue; and tissue from a region diametrically opposite to the affected region. Cores were also taken from healthy apples of comparable size from the same orchard and spraying regime. The results show that differences between 'opposite' and 'diseased' samples are seen at an incipient level in 'adjacent' samples but there is considerable apple to apple variation. NMR data has now been collected for similar 'opposite', 'adjacent', 'diseased', and 'healthy' cores for a much larger set of samples from 6 orchards and the data for analysis.

RNA was extracted from cortex tissue of Cox apples picked in June 2007 from trees that had been sprayed with triazole chemicals (fungicides and the growth regulator paclobutrazol) or no triazole chemicals. Initial real-time PCR studies have shown some differences in gene expression between treatments in some orchards but no consistent trends were observed. The range of target genes will be extended in future studies.

Since the anatomical investigations could not be started until the fruit samples came out of CA storage at the end of February 2008 there is nothing to report on this on-going part of the work.

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 TF166e 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 2007 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 (see also report on project TF 166h)
- As an interim measure growers have been advised that they should apply non-triazole fungicides and use alternative growth regulators such as prohexadione-calcium (‘Regalis’), particularly in orchards with a history of DBD
- 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.