

**Project Title:** Investigating diffuse browning disorder (DBD) in stored Cox apples

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EAST MALLING RESEARCH

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**Authentication**

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

.....D S Johnson  
Signature

Date .....

## **Grower summary**

### **Headlines**

Circumstantial evidence that low light levels during the growing season are conducive to diffuse browning disorder (DBD) development was not confirmed in an orchard trial where light availability was reduced by netting trees or increased by the use of the light reflecting product 'Extenday' laid in alleyways. No DBD occurred at EMR irrespective of Cultar regime. Similarly in a high-risk commercial orchard in Essex no DBD occurred in fruit from Cultar-treated or untreated areas although, in a high-risk commercial orchard in East Kent, DBD developed in fruit from Cultar-treated trees only.

### **Background and expected deliverables**

The purpose of the project is to identify the factors that induce diffuse browning disorder (DBD, previously known as either Boggy Bank disorder or Gorgate Syndrome) in Cox apples and to suggest measures that will prevent development of the disorder in CA-stored fruit.

Work done in year 1 of the project (1 April 2002 - 31 March 2003) focussed on the impact of storage conditions and post-storage temperature on the development of DBD. It is clear that little can be done to ameliorate the problem by modifying the storage atmosphere or temperature in consignments of Cox apples that are susceptible to the problem. However the time-course for development of DBD has confirmed preliminary advice to market stores of fruit at the first detection of symptoms. DBD is first seen in November but the number of affected fruits increases markedly from December through to February. Rigorous monitoring of Cox is essential, particularly of fruit from orchards with a history of DBD. It is especially important to subject fruit to a simulated 'shelf-life' in order to gauge the full extent of the problem. Indications are that post-storage temperature does not influence DBD development but that the removal of fruit from CA to air is the major factor that promotes further development of the disorder.

Clearly the root cause of the problem is associated with conditions that operate during the growing season that determine the susceptibility of the fruit to the disorder. There is little indication that mineral nutrition is implicated in the development of DBD.

Current indications are that there is a general association between climatic conditions during fruit development and the average seasonal risk of DBD. Low hours of sunshine, particularly in June and July, appear to heighten the risk of DBD although even in years of normal sunshine the disorder can occur in some orchards.

The work carried out in 2003-04 was intended to provide experimental evidence that the amount of light available to Cox trees during fruit development is a factor influencing susceptibility to DBD. On the basis of the circumstantial evidence that is available a broader hypothesis on the cause of DBD has been developed and other

experimental work done in 2003-04 was intended to test some aspects of this hypothesis.

One of the effects of low levels of light is the reduced synthesis of gibberellins (GA's). It is well documented that some of the GA biosynthesis enzymes are regulated by light. One of the possible impacts of low GA is a heightened susceptibility to senescence and it was interesting to note that fruit from the 3 orchards used in the 2002 study were highly susceptible to senescent breakdown in air storage and to DBD in CA storage. It has been suggested that the application of 'Cultar' (paclobutrazol) may be a contributing factor in the DBD problem. Although there is no clear evidence for this it would be consistent with the current hypothesis that 'Cultar' through its action in blocking gibberellin biosynthesis should increase susceptibility of fruit to DBD.

An experiment was carried out by FAST Ltd to test whether light levels are directly implicated in DBD development. This was achieved by increasing light into the tree using the light reflecting product 'Extenday' and by reducing light by the use of netting. This research complimented other trials to study the effects of 'Cultar' applied at East Malling and in high-risk commercial orchards.

## **Summary of project and main conclusions**

Two of the commercial orchards with high risk of DBD that were used in the previous year were selected for an evaluation of the effect of 'Cultar' on the development of the disorder. In the East Kent orchard (ref. TC) 3 rows (approximately 150 trees) were not treated with 'Cultar' and in the Essex orchard (ref. FH) 2 rows (approximately 100 trees) were left untreated. Samples of fruit for storage were taken on 5 (TC) and 8 (FH) September 2003 and transported immediately to the Jim Mount Building at East Malling. In order to obtain representative samples from the treated and untreated areas 3 or 4 fruits were sampled from every tree in the treated rows and trees in the 'Cultar'-treated areas were sampled in the same way. One or two rows adjacent to the untreated rows acted as guard rows and were not sampled. During sampling fruits were selected at random at about waist height and from both sides of the trees. On arrival at East Malling the fruit from the 'Cultar'-treated and untreated areas were randomised to form samples for storage in air at 1.5-2°C and 3-3.5°C and in CA (1.2% O<sub>2</sub>, <1% CO<sub>2</sub>) at 3.5-4°C. Further samples were taken for an assessment of fruit maturity and for mineral analysis.

A 4-year-old Queen Cox orchard (Plot EE191) at EMR was used for an experiment which comprised two Cultar treatments and an untreated control. Cultar was applied on 6 or 13 occasions at 0.5 and 0.23 L ha<sup>-1</sup> respectively. Both programs commenced on 5 June 2003 and the total Cultar applied during the growing season was the same (3 L ha<sup>-1</sup>). Sprays were applied at weekly intervals and finished on 11 July 2003 (6-spray program) or 1 September 2003 (13-spray program). The trees were picked on 11 September 2003 and the entire crop from each tree was weighed and counted. Sub-samples of fruit were taken for storage in CA (1.2% O<sub>2</sub>, <1% CO<sub>2</sub>) at 3.5-4°C. Fruit was removed from store on 17 February 2004 and, after weighing, 10 fruits were removed from each sample for measurement of background colour and firmness prior to an internal examination of the fruit for the presence of DBD and other

physiological disorders. The remaining fruit in the samples were kept for a further 7 days at 20°C and then examined again for internal defects.

A trial was carried out by FAST Ltd in a commercial orchard of Queen Cox situated in North Kent (Lynsted). The orchard was planted in 1994/1995 at a spacing of 3 x 1.2m and had a history of DBD problems in the stored fruit. The trial was designed to provide two areas where the light reflecting product 'Extenday' was laid down in the alleyways between the trees and two similar areas where there was no 'Extenday'. Within each of the four main areas there were 2 plots that compared shaded and un-shaded trees. There were 4 shaded and 4 un-shaded trees within each plot. Shading was provided by covering the top third of the canopy with netting (HPN 109/50 Mark VI Green 3.2m wide from Lows of Dundee) that was intended to reduce light penetration by 50%. The netting was erected on 2 June 2003 and remained in place until harvest. On 9 July 2003 light levels (photosynthetically active radiation or PAR) were measured above and within the canopy of the trees and in the alleyways using an AccuPAR PAR Ceptometer (Decagon Devices Inc.). Samples of mid-extension shoot leaves were taken for mineral analysis two weeks before harvest. The experimental trees were harvested on 9 and 10 September 2003 and sub-samples for storage were transported immediately to the post harvest facility (Jim Mount Building) at East Malling Research. Additional samples were taken by F.A.S.T. Ltd for maturity assessment, grading for size and red colour and mineral analysis. Fruit was removed from CA storage (1.2% O<sub>2</sub>, <1% CO<sub>2</sub> at 3.5-4°C) on 18 February 2004 and, after weighing, 10 fruits were removed from each sample for measurement of background and red colour and firmness prior to an internal examination of the fruit for the presence of DBD and other physiological disorders. A second set of samples were kept for a further 7 days at 20°C and then examined for internal defects.

## **Effects of Cultar**

Application of Cultar in commercial orchards had no effect on maturity parameters at harvest but resulted in smaller fruit that were higher in calcium and tended to be higher in potassium and phosphorus. Cultar-treated fruit stored in air until November and in CA until February were firmer than untreated fruit. The effect of Cultar was greater in fruit from the Essex orchard where the firmness benefit was commercially significant. DBD developed in fruit from the East Kent orchard only in fruit from the area in the orchard that had received Cultar sprays. The development of DBD in Cultar-treated fruit kept in CA storage corresponded with the development of senescent breakdown in similar samples kept in air storage until January. This is consistent with results obtained in the first year of the investigation.

In the trial at EMR there were no significant effects of Cultar treatments on fruit yield at harvest or on weight loss, background colour and firmness of CA-stored fruit. No DBD was found irrespective of Cultar use.

## Effect of Shading and Extenday

Shading resulted in a 41% reduction in PAR (photosynthetically active radiation) under the top surface of the nets and at waist height in the canopy shading reduced PAR by about 50%. 'Extenday' reflected additional light to the sensors positioned in the alleyways and increased the amount of light reflected into the lower canopy by about 50%.

Since dry matter production of a plant canopy is directly related to the amount of photosynthetically useful radiation intercepted it was expected that fruit yield and fruit size would be reduced by the shading applied. Shading resulted in a significant reduction in yield (by 25%) but there was no significant increase in yield by the use of 'Extenday'. Fruit number and mean fruit weight were highly variable from tree to tree and there were no significant effects of shading or 'Extenday' treatments on these parameters. However, size grade data indicated a higher proportion of apples in the lower size ranges from shaded trees without 'Extenday'.

Shading resulted in lower starch coverage and the background colour tended to be greener. In the plot without 'Extenday' shading resulted in firmer fruit. Clearly shading has a direct effect on the parameters normally used to judge maturity of fruits and their suitability for storage. The reduced starch coverage in fruit from shaded trees is likely to result from carbohydrate shortage rather than an indication of advanced maturity. There was a marked effect of shading on the red colour grade-out. Since red colour development in apple skin is directly dependent on light it was not surprising that shading increased the proportion of poorly coloured apples.

There were no effects of 'Extenday' on the mineral composition of leaves but shading increased the concentration of P, K, K/Ca, B, Zn, and Fe and tended to increase Cu and Mg. Shading increased the Ca concentration in fruits from plots not covered with 'Extenday' and generally tended to increase Mg concentrations.

The quality of fruits removed from CA storage on 18 February was affected by shading but not by 'Extenday'. Shaded fruits tended to be smaller and firmer and were significantly less red with a greener background colour.

Internal examination of the fruit revealed a range of browning disorders that may or may not be related. A high percentage of fruits were affected by classical symptoms of DBD but additionally in some fruits DBD was insufficiently defined. Consequently a possible and definite category was applied to the fruits. Additionally in some fruit there was a general browning in the calyx region whilst others showed classic symptoms of senescent breakdown.

Although shading reduced the percentage of fruit with possible DBD and 'Extenday' reduced the percentage of fruit with definite DBD, in general there was no effect of treatments on calyx browning or DBD-like symptoms. Senescent breakdown failed to develop in shaded fruit which is likely to be related to higher concentrations of Ca in the fruit.

## **Conclusions**

Circumstantial evidence that DBD is encouraged by low light conditions was supported by the low incidence of the problem in commercial fruit produced in the 'high-light' year of 2003. Shading trees to reduce light availability did not exacerbate 'definite' DBD but increasing reflected light by the use of 'Extenday' reduced the percentage of fruit affected by classic DBD symptoms. In an East Kent orchard with a history of DBD the application of Cultar was associated with DBD development in 2003-4 but no DBD occurred in fruit from a high risk orchard in Essex or in an orchard at EMR irrespective of Cultar use.

The experimental results did not support the hypothesis of a direct involvement of light in predisposing fruit to DBD but clearly the significant amount of DBD that developed in the fruit from the orchard used in this experiment confirms an orchard factor influence in the problem.

In 2004-05 it is intended to continue our research to test the hypothesis that DBD is the result of reduced gibberellin synthesis in the fruit during its development on the tree. We will continue with experiments to evaluate the effect of light availability in orchards (with collaboration of FAST Ltd) and determine the influence of Cultar application. In addition it is proposed to evaluate the effects of pre-harvest sprays of gibberellic acid on DBD development in fruit from high-risk orchards.

## **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 in the first year was helpful in providing advice on how to manage crops of fruit from orchards with a history of DBD. These action points are repeated below. No additional actions points have arisen as a result of the work done in year 2 of the project.



## Action points for growers

- Where fruit from orchards with a history of diffuse browning disorder (DBD) are stored in CA they should be kept at a minimum temperature of 4°C and marketed early.
- Growers can use either 1.2 or 2% O<sub>2</sub> (<1%CO<sub>2</sub>) for the storage of Cox regardless of DBD risk. It is not known whether storage in 5%CO<sub>2</sub> + 3%O<sub>2</sub> would affect the development of DBD.
- Growers should keep themselves aware of the perceived risk of DBD reported by advisory groups.
- Where there is no perceived risk of DBD it is important to adhere rigidly to minimum recommended storage temperatures (3.5-4°C) for CA storage.
- Regardless of DBD risk fruit stored in air should be kept at 1.5°C and sold by mid-October.
- Growers should monitor fruit rigorously. Examine the calyx region of the fruit carefully and repeat the examination after a further 7 days at room temperature (18-20°C).
- Detection of DBD should prompt immediate marketing.

## Science Section

### Introduction

Work done in year 1 of the project (1 April 2002 - 31 March 2003) focussed on the impact of storage conditions and post-storage temperature on the development of DBD. Preliminary results were presented at the EMRA/MFSS Fruit Storage Day held at EMR on 27 March 2003. It is clear that little can be done to ameliorate the problem by modifying the storage atmosphere or temperature in consignments of Cox apples that are susceptible to the problem. However the time-course for development of DBD has confirmed preliminary advice to market stores of fruit at the first detection of symptoms. DBD is first seen in November but the number of affected fruits increases markedly from December through to February. Rigorous monitoring of Cox is essential, particularly of fruit from orchards with a history of DBD. It is especially important to subject fruit to a simulated 'shelf-life' in order to gauge the full extent of the problem. Indications are that post-storage temperature does not influence DBD development but that the removal of fruit from CA to air is the major factor that promotes further development of the disorder.

Clearly the root cause of the problem is associated with conditions that operate during the growing season that determine the susceptibility of the fruit to the disorder. There is little indication that mineral nutrition is implicated in the development of DBD. Over 50 individual apples (affected and unaffected) from the orchards used in the 2002 study were analysed for major (N, P, K, Ca and Mg) and minor / trace elements (B, Cu Zn and Mn). Preliminary analyses of the data indicate no differences in mineral levels in affected and unaffected fruit.

Current indications are that there is a general association between climatic conditions during fruit development and the average seasonal risk of DBD. Low hours of sunshine, particularly in June and July, appear to heighten the risk of DBD although even in years of normal sunshine the disorder can occur in some orchards.

The work carried out in 2003-04 was intended to provide experimental evidence that the amount of light available to Cox trees during fruit development is a factor influencing susceptibility to DBD. On the basis of the circumstantial evidence that is available a broader hypothesis on the cause of DBD has been developed and other experimental work done in 2003-04 was intended to test some aspects of this hypothesis.

One of the effects of low levels of light is the reduced synthesis of gibberellins (GA's). It is well documented that some of the GA biosynthesis enzymes are regulated by light. One of the possible impacts of low GA is a heightened susceptibility to senescence and it was interesting to note that fruit from the 3 orchards used in the 2002 study were highly susceptible to senescent breakdown in air storage and to DBD in CA storage. It has been suggested that the application of 'Cultar' (paclobutrazol) may be a contributing factor in the DBD problem. Although there is no clear evidence for this it would be consistent with the current hypothesis that 'Cultar' through its action in blocking gibberellin biosynthesis should increase susceptibility of fruit to DBD.

An experiment was carried out by FAST Ltd to test whether light levels are directly implicated in DBD development. This was achieved by increasing light into the tree using the light reflecting product 'Extenday' and by reducing light by the use of netting. This research complimented other trials to study the effects of 'Cultar' applied at East Malling and in high-risk commercial orchards.

## **Materials and Methods**

### *Cultar application in commercial orchards*

Two of the orchards with high risk of DBD that were used in the previous year were selected for an evaluation of the effect of 'Cultar' on the development of the disorder. In the East Kent orchard (ref. TC) 3 rows (approximately 150 trees) were not treated with 'Cultar' and in the Essex orchard (ref. FH) 2 rows (approximately 100 trees) were left untreated. Samples of fruit for storage were taken on 5 (TC) and 8 (FH) September 2003 and transported immediately to the Jim Mount Building at East Malling. In order to obtain representative samples from the treated and untreated areas 3 or 4 fruits were sampled from every tree in the treated rows and trees in the 'Cultar'-treated areas were sampled in the same way. One or two rows adjacent to the untreated rows acted as guard rows and were not sampled. During sampling fruits were selected at random at about waist height and from both sides of the trees. On arrival at East Malling the fruit from the 'Cultar'-treated and untreated areas were randomised to form samples for storage in air at 1.5-2°C and 3-3.5°C and in CA (1.2% O<sub>2</sub>, <1% CO<sub>2</sub>) at 3.5-4°C. Further samples were taken for an assessment of fruit maturity and for mineral analysis. Maturity was assessed by measuring internal ethylene concentration, firmness, soluble solids concentration and starch content of the fruit as described below. A commercial analytical laboratory (NRM Ltd) carried out mineral analysis.

Internal ethylene concentration (IEC). IEC was measured on 5 intact undamaged apples from each replicate of each treatment. A sample of the internal atmosphere of each apple was taken by syringe (0.5ml) and injected into a gas chromatograph fitted with an alumina column and FID detector. Results were expressed as parts per billion (ppb) of ethylene.

Fruit firmness. Two measurements were made on the opposite sides of each fruit using an LRX (Lloyd Instruments) materials testing machine fitted with an 11mm probe. Measurements were made in the equatorial region after removal of the peel. Firmness was the maximum force (N) recorded during the insertion of the probe to a depth of 8mm.

Soluble solids concentration. Juice was extracted from each apple using a 'Chylofel' (Copa - Technologie S.A.) apparatus and mixed to form a composite sample. Soluble solids concentration (%) was measured using a BRX-242 refractometer (Camlab Ltd).

Starch test. Half of each apple cut for internal examination was dipped in a solution containing 0.1% w/v iodine and 4% w/v potassium iodide. Dipped sections were left for at least an hour before being assessed. Each apple was scored (1-slight central discoloration to 10-no peripheral discoloration) using the starch conversion chart for

apples (circular type) issued by Ctifl. An average score was calculated for each sample.

#### *Cultar application at EMR*

A 4-year-old Queen Cox orchard (Plot EE191) at EMR was used for the experiment which comprised two Cultar treatments and an untreated control. Cultar was applied on 6 or 13 occasions at 0.5 and 0.23 L ha<sup>-1</sup> respectively. Both programs commenced on 5 June 2003 and the total Cultar applied during the growing season was the same (3 L ha<sup>-1</sup>). Sprays were applied at weekly intervals and finished on 11 July 2003 (6-spray program) or 1 September 2003 (13-spray program). The trees were picked on 11 September 2003 and the entire crop from each tree was weighed and counted. Sub-samples of fruit were taken for storage in CA (1.2% O<sub>2</sub>, <1% CO<sub>2</sub>) at 3.5-4°C. Fruit was removed from store on 17 February 2004 and, after weighing, 10 fruits were removed from each sample for measurement of background colour and firmness prior to an internal examination of the fruit for the presence of DBD and other physiological disorders. The remaining fruit in the samples were kept for a further 7 days at 20°C and then examined again for internal defects. The method for measurement of fruit firmness was as described previously. Background colour was measured according to the method described below.

Background colour. The colour of the non-blush side of the fruit was assessed using commercial (World Wide Fruit / Qualytech) colour charts. Background colour of each fruit was compared against 4 cards that range from green (1) to yellow (4). The average score was calculated for each sample.

#### *Shading / Extenday trial in a commercial orchard*

A trial was carried out by FAST Ltd in a commercial orchard of Queen Cox situated in North Kent (Lynsted). The orchard was planted in 1994/1995 at a spacing of 3 x 1.2m and had a history of DBD problems in the stored fruit. A trial was designed to provide two areas where the light reflecting product 'Extenday' was laid down in the alleyways between the trees and two similar areas where there was no 'Extenday'. Within each of the four main areas there were 2 plots that compared shaded and unshaded trees. There were 4 shaded and 4 unshaded trees within each plot. Shading was provided by covering the top third of the canopy with netting (HPN 109/50 Mark VI Green 3.2m wide from Lows of Dundee) that was intended to reduce light penetration by 50%. The netting was erected on 2 June 2003 and remained in place until harvest. On 9 July 2003 light levels (photosynthetically active radiation or PAR) were measured above and within the canopy of the trees and in the alleyways using an AccuPAR PAR Ceptometer (Decagon Devices Inc.). Samples of mid-extension shoot leaves were taken for mineral analysis two weeks before harvest. The experimental trees were harvested on 9 and 10 September 2003 and sub-samples for storage were transported immediately to the post harvest facility (Jim Mount Building) at East Malling Research. Additional samples were taken by FAST Ltd for maturity assessment (starch pattern, firmness, soluble solids concentration, Streif Index and background colour), grading for size and red colour and mineral analysis. Fruits (25 per tree) were graded for size into 5mm diameter size bands (range <40 to >75 mm) and the amount of red colour (percentage of the surface area) on each apple was assessed and recorded in 5% colour bands (range 0-100%). Details of the maturity

measurements are described below. Fruit was removed from CA storage (1.2% O<sub>2</sub>, <1% CO<sub>2</sub> at 3.5-4°C) on 18 February 2004 and, after weighing, 10 fruits were removed from each sample for measurement of background and red colour and firmness (LRX instrument) prior to an internal examination of the fruit for the presence of DBD and other physiological disorders. A second set of samples was kept for a further 7 days at 20°C and then examined for internal defects. Leaf and fruit analysis was carried out by FAST Ltd.

Background colour. Assessed using commercial (World Wide Fruit / Qualytech) colour charts as described previously.

Fruit firmness. Two measurements were made on the opposite sides of each fruit using an 'Effigi' penetrometer mounted in a drill-stand and fitted with an 11mm probe. Measurements were made in the equatorial region after removal of the peel. Firmness was the maximum force (kg) recorded during the insertion of the probe to a depth of 8mm.

Soluble solids concentration. Juice was extracted from each apple using a steel rod and the soluble solids concentration (%) was measured using a hand-held refractometer.

Starch test. Half of each apple was dipped in a solution containing 0.1% w/v iodine and 4% w/v potassium iodide as described previously. The percentage of the cut surface stained black was estimated with the aid of transparent sheets printed with a series of gauges (concentric rings of decreasing radii) (Cockburn and Sharples, 1979).

Streif maturity index. Calculated by dividing the firmness value expressed in Newtons (kg\*9.81) by the product of the soluble solids concentration (%) and starch cover (%) converted to a score between 1 (completely black) and 10 (completely white).

### *Statistical analysis*

Data from the replicated field trials was subjected to analysis of variance (ANOVA) using GENSTAT 6 statistical software. The overall effects of treatments can be compared using the standard errors of the difference between means (s.e.d.) and degrees of freedom (d.f.) given in the tables. Percentage data from the shading / Extenday trial were transformed to angles prior to analysis.

## **Results and Discussion**

### *Cultar application in commercial orchards*

It was not possible to apply statistical tests to the data as it was not practicable to replicate the treatments in the grower's orchards. Application of Cultar had no effect on maturity parameters at harvest (Table 1) but resulted in smaller fruit that were higher in calcium and tended to be higher in potassium and phosphorus (Table 2). Cultar-treated fruit stored in air until November and in CA until February were firmer than untreated fruit (Table 4). The effect of Cultar was greater in fruit from the Essex orchard where the firmness benefit was commercially significant. The benefit of

reducing the storage temperature for air-stored Cox was also confirmed. DBD developed in fruit from the East Kent orchard but only in fruit from the area in the orchard that had received Cultar sprays (Table 5). The development of DBD in Cultar-treated fruit kept in CA storage corresponded with the development of senescent breakdown in similar samples kept in air storage until January. This is consistent with results obtained in the first year of the investigation.

**Table 1.** Maturity parameters of Cox apples sampled from areas in orchards in E. Kent (TC) and Essex (FH) that were sprayed or not sprayed with Cultar.

Site	Pick date	Cultar	Internal ethylene conc.		Firmness (N)	Soluble solids (%)	Starch (1,black - 10,white)
			(ppb)	% > 100 ppb			
TC	5.9.03	No	215	50	89.9	11.0	4.0
		Yes	224	50	88.5	11.0	3.4
FH	8.9.03	No	151	70	99.6	12.4	2.5
		Yes	192	80	99.2	12.4	2.1

**Table 2.** Analysis of major minerals (mg 100g<sup>-1</sup>) in bulk samples of fruit at harvest in 2003 and standards for Cox stored in CA conditions of 1.2% O<sub>2</sub>, <1% CO<sub>2</sub> at 3.5-4°C.

Site	Cultar	Mean Fruit Wt (g)	N	P	K	Ca	Mg	K/Ca
TC	No	171.7	68	9.9	124	3.3	5.8	37.3
	Yes	162.3	64	10.4	132	3.8	5.7	34.5
FH	No	137.6	43	9.6	114	4.6	5.2	24.6
	Yes	127.5	43	9.9	120	5.0	5.5	23.9
Standards for CA			50-70	11 min	130-150	4.5	5	35

**Table 3.** Analysis of minor and trace minerals (mg 100g<sup>-1</sup>) in bulk samples of fruit at harvest in 2003.

Site	Cultar	B	Cu	Mn	Zn
TC	No	0.27	0.045	0.039	0.038
	Yes	0.29	0.046	0.036	0.049
FH	No	0.31	0.040	0.033	0.053
	Yes	0.34	0.042	0.033	0.030

**Table 4.** Firmness (N) and background colour (1, green-4, yellow) of Cox apples sampled from areas in orchards in E. Kent (TC) and Essex (FH) that were sprayed or not sprayed with Cultar and stored in air at 1.5°C and 3°C and in CA (<1% CO<sub>2</sub> + 1.2% O<sub>2</sub>) at 3.5°C.

Site	Pick date	Cultar	Ground colour					Firmness	
			CA Feb.	Air November		CA			
				1.5°C	3°C	Nov.	Feb.		
TC	5.9.03	No	1.6	57.5	48.9	81.4	65.7		
		Yes	1.8	58.6	49.9	80.5	70.8		
FH	8.9.03	No	1.8	57.3	54.0	90.3	68.6		
		Yes	1.7	66.5	58.8	90.5	81.1		

**Table 5.** Physiological storage disorders, bitter pit (BP), senescent breakdown (SB) and diffuse browning disorder (DBD) in Cox apples sampled from areas in orchards in E. Kent (TC) and Essex (FH) that were sprayed or not sprayed with Cultar.

Site	Pick date	Cultar	Air storage 3°C until 12.1.04			CA (<1% CO <sub>2</sub> + 1.2% O <sub>2</sub> ) storage at 3.5°C until 16.2.04 + 7d at 20°C		
			BP %	SB %	DBD %	BP %	SB %	DBD %
TC	5.9.03	No	70.0	12.5	0	2.5	5.0	0
		Yes	53.5	34.9	2.3	4.2	4.2	14.6
FH	8.9.03	No	12.5	0	0	0	7.5	0
		Yes	12.5	0	0	0	5.0	0

#### *Cultar application at EMR*

Apples from trees sprayed on 6 occasions with Cultar tended to have a lower mean weight than the untreated or those from trees sprayed on 13 occasions but the effect failed to reach significance at the 5% level (Table 6). There were no significant effects of Cultar treatments on fruit yield at harvest or on weight loss, background colour and firmness of CA-stored fruit. No DBD was found irrespective of Cultar use.

**Table 6.** Effect of ‘Cultar’ sprays (6 at 0.5L ha<sup>-1</sup> or 13 at 0.23L ha<sup>-1</sup>) on the yield (weight and number of fruit per tree) and mean weight of Queen Cox apples harvested on 12 September 2003 and on the weight loss, ground colour and firmness of fruit after CA storage (1.2% O<sub>2</sub> + <1% CO<sub>2</sub>) at 3.5°C until 17 February 2004.

	Yield tree <sup>-1</sup> (kg)	Fruit No. tree <sup>-1</sup>	Mean Fruit Wt (g)	Wt loss (%)	Ground Colour (1= green 4= yellow)	Firmness (N)
Unsprayed	6.94	60	119.5	1.3	1.9	66.4
Cultar x6	6.93	70	97.8	1.0	1.9	67.9
Cultar x13	6.48	58	123.1	1.3	2.1	66.1
s.e.d. (10 d.f.)	1.650	16.3	10.35	0.23	0.11	2.26

#### *Shading / Extenday trial in a commercial orchard*

PAR measurements were carried out during the morning of 9 July 2003 under bright sunny conditions. The results presented in Table 7 are from simultaneous measurements of PAR above and within the tree canopy. Shading resulted in a 41% reduction in PAR under the top surface of the nets and at waist height in the canopy shading reduced PAR by about 50%. 'Extenday' reflected additional light to the sensors positioned in the alleyways and increased the amount of light reflected into the lower canopy by about 50%.

Since dry matter production of a plant canopy is directly related to the amount of photosynthetically useful radiation intercepted it was expected that fruit yield and fruit size would be reduced by the shading applied. Shading resulted in a significant reduction in yield (by 25%) but there was no significant increase in yield by the use of 'Extenday' (Table 8). It should be noted that any potential effects of 'Extenday' would be more difficult to prove statistically in view of the limited replication. From a practical perspective Extenday had to be applied over a large area which limited the possibilities for replication. In this case with only 2 areas of with and without 'Extenday' the error term in the analysis of variance was likely to be high. Fruit number and mean fruit weight were highly variable from tree to tree and there were no significant effects of shading or 'Extenday' treatments on these parameters. However, size grade data indicated a higher proportion of apples in the lower size ranges from shaded trees without 'Extenday' (Fig. 2).

Maturity measurements were carried out on only half the number of the plots and consequently it was not possible to carry out a statistical analysis of the effects of 'Extenday'. Shading resulted in lower starch coverage and the background colour tended to be greener (Table 8). In the plot without 'Extenday' shading resulted in firmer fruit (interactive effects not shown). Clearly shading has a direct effect on the parameters normally used to judge maturity of fruits and their suitability for storage. The reduced starch coverage in fruit from shaded trees is likely to result from carbohydrate shortage rather than an indication of advanced maturity. There was a marked effect of shading on the red colour grade-out. Since red colour development in apple skin is directly dependent on light it was not surprising that shading increased the proportion of poorly coloured apples (Fig. 1).

There were no effects of 'Extenday' on the mineral composition of leaves but shading increased the concentration of P, K, K/Ca, B, Zn, and Fe and tended to increase Cu and Mg (Table 9). It is known that shading reduces total growth in apple trees and it is possible that minerals taken up by the shoots of shaded trees would be concentrated by reduced growth.

Shading increased the Ca concentration in fruits from plots not covered with 'Extenday' and generally tended to increase Mg concentrations (Table 10). It is generally observed that shading results in fruits that are smaller and weigh less. However, in this trial the effect of shade on mean fruit weight was not statistically significant (Table 8). Sub-samples taken for storage showed a reduced mean weight in fruit from shaded trees but again the effect failed to reach statistical significance (Table 11).



The quality of fruits removed from CA storage on 18 February was affected by shading but not by 'Extenday' (Table 11). Shaded fruits tended to be smaller and were significantly less red with a greener background colour. Shaded fruits tended to be firmer although the effect just failed to reach significance at the 5% level.

Internal examination of the fruit revealed a range of browning disorders that may or may not be related. A high percentage of fruits were affected by classical symptoms of DBD but additionally in some fruits DBD was insufficiently defined. Consequently a possible and definite category was applied to the fruits (Table 12). Additionally in some fruit there was a general browning in the calyx region whilst others showed classic symptoms of senescent breakdown.

Although shading reduced the percentage of fruit with possible DBD and Extenday reduced the percentage of fruit with definite DBD, in general there was no effect of treatments on calyx browning or DBD-like symptoms. Senescent breakdown failed to develop in shaded fruit which is likely to be related to higher concentrations of Ca in the fruit (Table 10).

Circumstantial evidence that DBD is encouraged by low light conditions was supported by the low incidence of the problem in commercial fruit produced in the 'high-light' year of 2003. Shading trees to reduce light availability did not exacerbate 'definite' DBD but increasing reflected light by the use of 'Extenday' reduced the percentage of fruit affected by classic DBD symptoms.

The experimental results did not support the hypothesis of a direct involvement of light in predisposing fruit to DBD but clearly the significant amount of DBD that developed in the fruit from the orchard used in this experiment confirms an orchard factor influence in the problem.

**Table 7.** Effects of shading and 'Extenday' on the levels of photosynthetically active radiation (PAR) compared to above canopy levels measured on 9 July 2003.

	% of PAR measured above the canopy			
	Sensor horizontal		Sensor vertical	
	Under top of nets	Waist high in the canopy	In canopy ground to waist high	In alley ground to waist high
Shade	41.0	22.9	6.3	-
No Shade	-	40.2	5.7	-
Extenday	41.7	33.5	7.9	86.4
No Extenday	40.3	29.6	4.1	60.5

**Table 8.** The effects of shading and ‘Extenday’ treatments on yield (number and weight of fruit per tree) and maturity parameters of Queen Cox apples at harvest.

	Extenday			Shade		
	Yes	No	s.e.d.	No	Yes	s.e.d.
Yield kg tree <sup>-1</sup>	12.42	10.55	1.707	13.12	9.85*	0.917
Number tree <sup>-1</sup>	130	117	20.5	132	116	19.0
Mean Fruit Wt (g)	98.1	92.6	4.61	101.8	89.0	11.02
Starch (%)	45	56	-	57	44*	2.07
Firmness (kg)	8.2	8.4	-	8.3	8.4	0.08
Sol. Solids (%)	13.5	14.1	-	13.9	13.7	0.23
Streif Index	1.2	1.3	-	1.3	1.1	0.04
Ground Colour	1.8	1.8	-	1.9	1.7	0.04

(\* indicates a significant treatment effect at the 5% level of probability)

**Table 9.** The effects of shading and ‘Extenday’ treatments on the mineral composition of extension shoot leaves sampled from Queen Cox trees.

	Extenday		s.e.d. (2 df)	Shade		s.e.d. (6 df)
	Yes	No		No	Yes	
N (%)	2.6	2.7	0.14	2.8	2.5	0.20
P (%)	0.24	0.22	0.011	0.22	0.24*	0.009
K (%)	1.3	1.2	0.04	1.0	1.4**	0.08
Ca (%)	1.5	1.5	0.13	1.5	1.5	0.07
Mg (%)	0.15	0.14	0.024	0.13	0.16	0.014
K/Ca	0.85	0.79	0.083	0.67	0.97**	0.062
Mn (mg/kg)	41.5	29.5	12.75	35.6	35.4	1.92
B (mg/kg)	18.1	17.7	0.26	16.8	19.0***	0.18
Cu (mg/kg)	6.9	6.0	0.37	5.9	7.0	0.48
Zn (mg/kg)	13.9	13.6	0.52	12.4	15.0*	0.79
Fe (mg/kg)	111	111	10.1	94	129**	8.7

(\* \*\* and \*\*\* indicates a significant treatment effect at the 5, 1 and 0.1% level of probability respectively)

**Table 10.** The effects of shading and ‘Extenday’ treatments on the mineral composition of Queen Cox apples sampled at harvest.

	Extenday		s.e.d. (2 df)	Shade		s.e.d. (6 df)
	Yes	No		No	Yes	
N (mg/100g)	57.2	53.8	7.57	55.6	55.4	4.50
P (mg/100g)	13.0	11.9	1.51	12.0	12.9	0.59
K (mg/100g)	162	155	11.6	158	159	6.6
Ca (mg/100g)	7.3	7.8	0.66	7.2	7.9*	0.27
Mg (mg/100g)	6.8	6.7	0.64	6.5	6.9	0.17
K/Ca	22.3	20.3	2.57	22.1	20.4	1.45
Mn (mg/kg)	0.51	0.48	0.053	0.48	0.51	0.022
B (mg/kg)	2.2	2.5	0.51	2.5	2.3	0.54
Zn (mg/kg)	0.44	0.38	0.103	0.41	0.42	0.117
Fe (mg/kg)	1.8	1.6	0.29	1.6	1.7	0.18

(\* indicates a significant treatment effect at the 5% level of probability)

**Table 11.** The effects of shading and ‘Extenday’ treatments on the size and quality of Queen Cox apples stored in 1.2% O<sub>2</sub> + <1% CO<sub>2</sub> at 3.5°C until 18 February 2004.

	Mean Fruit weight (g)	Ground colour (1= green, 4= yellow)	Red Colour (5 = 100% cover)	Firmness (N)
Shade	101.5	1.70*	2.88**	65.3
No Shade	113.1	2.10	3.93	60.9
s.e.d (6 d.f.)	6.58	0.120	0.249	1.96
Extenday	116.5*	1.91	3.55	63.7
No Extenday	98.1	1.89	3.25	62.5
s.e.d (2 d.f.)	4.34	0.128	0.340	4.47

(\* and \*\* indicates a significant treatment effect at the 5% and 1% level of probability respectively)

**Table 12.** The effects of shading and ‘Extenday’ treatments on the development of physiological disorders in Queen Cox apples stored in 1.2% O<sub>2</sub> + <1% CO<sub>2</sub> at 3.5°C until 18 February 2004. Data are means for fruit examined immediately ex-store and after a further 7 days at 20°C. Percentage data were converted to angles prior to analysis.

	Calyx Browning (%)	Diffuse Browning Disorder (%)		All DBD-like symptoms (%)	Senescent (cortex) Breakdown (%)
		Possible	Definite		
Shade	10.4	9.8*	12.1	20.4	0*
No Shade	12.6	17.1	11.6	26.0	7.1
s.e.d (6 d.f.)	2.61	2.69	3.90	4.19	3.07
Extenday	11.9	15.0	10.2*	23.5	3.1
No Extenday	11.1	11.9	13.4	22.9	4.0
s.e.d (2 d.f.)	4.30	3.69	0.65	4.92	5.09

(\* indicates a significant treatment effect at the 5% level of probability)

Figure 1. The effects of shading and 'Extenday' treatments on red colour distribution in Queen Cox apples at harvest.

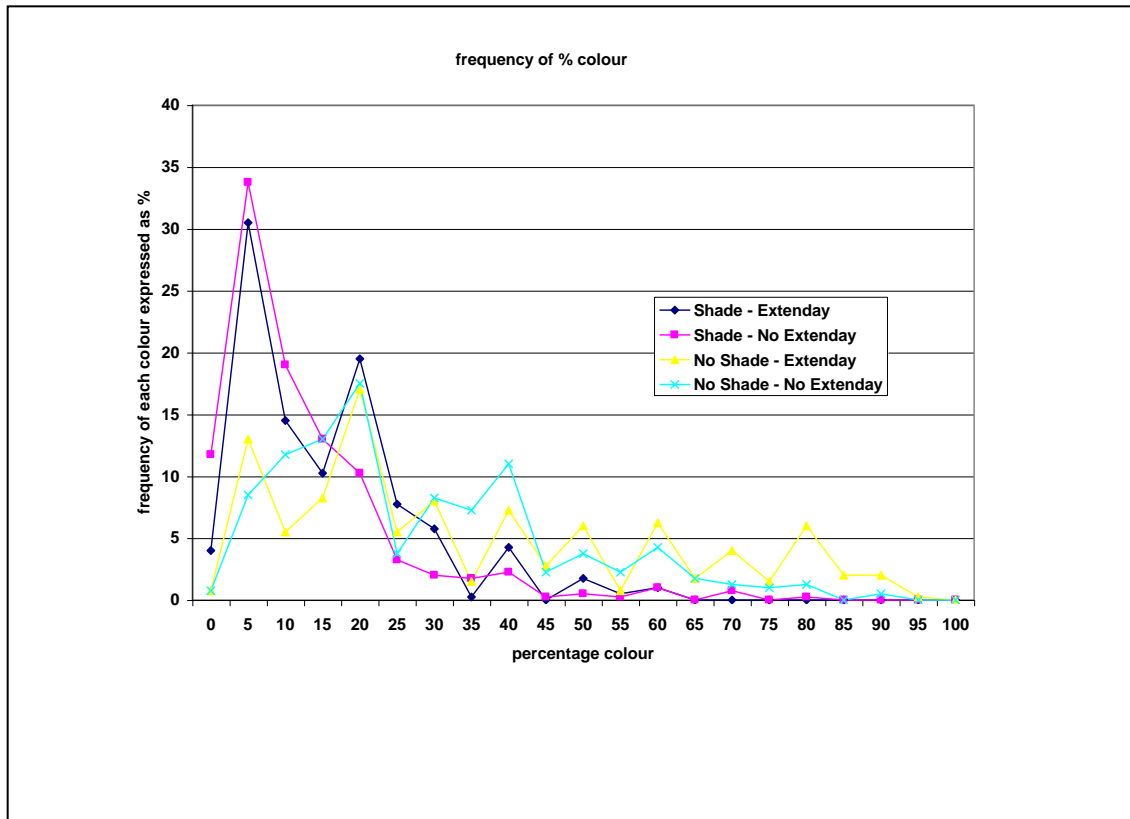
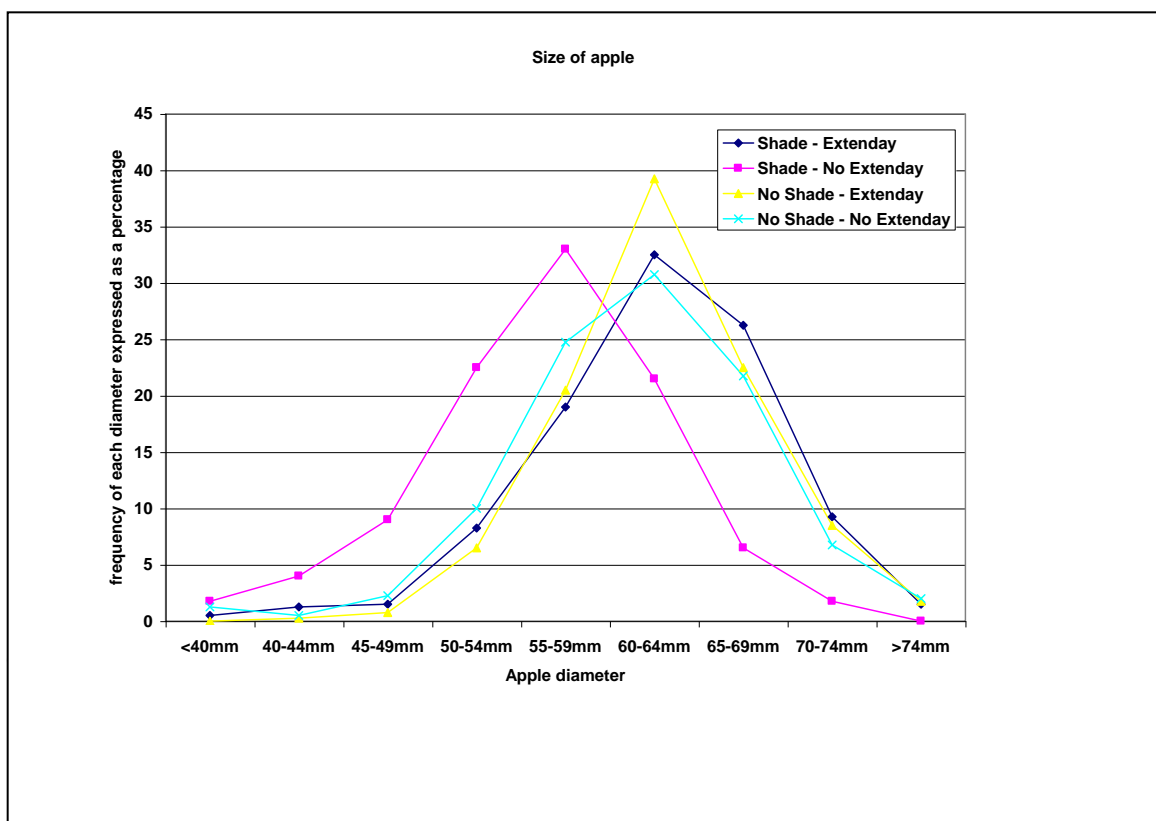


Figure 2. The effects of shading and 'Extenday' treatments on size distribution in Queen Cox apples at harvest.



The working hypothesis at the beginning of the second year of the project was that low light levels during the growing season may predispose Cox apples to DBD by reducing gibberellin levels in the fruit. The use of growth control chemicals such as 'Cultar' could encourage DBD development by blocking gibberellin biosynthesis.

The results obtained in the various experiments in 2003-4 have not generally supported the hypothesis. In a trial on young trees at EMR no DBD was found in stored fruit irrespective of the Cultar program applied. Similarly DBD did not develop in Cultar-sprayed or unsprayed fruit from a commercial orchard in Essex that had a history of the problem. However, in fruit stored from an East Kent orchard with a history of DBD problems the disorder developed in fruit from Cultar-sprayed trees only. Although it is clear that Cultar use is not the cause of DBD in Cox it cannot be ruled out as a contributing factor. Seasonal and orchard factors are clearly implicated in DBD susceptibility. The hypothesis proposed at the beginning of year 2 of the project was that low gibberellin concentrations in apple fruits may be contributing to the problem. This was based on circumstantial evidence that DBD was worse in dull growing seasons and the potential involvement of gibberellin inhibitors for growth control. The low numbers of reports of DBD in commercial fruit in 2003-4 supports the hypothesis that light level in orchards is a critical factor. The 2003 growing season will be remembered for achieving record temperatures. However, attempts to confirm the influence of light by the use of shading to reduce light penetration into the tree canopy and of 'Extenday' to increase light reflection from the alleyways into the trees were not successful. Although, as expected, shading resulted in lower yields of smaller and less coloured fruit it did not influence the incidence of DBD-like symptoms in the stored fruit. Although 'Extenday' increased light in the lower parts of the tree canopy and tended to increase fruit yield, size and colour (although not significantly so) it did not affect the percentage of stored fruit affected by DBD-like symptoms.

In 2004-05 it is intended to continue our research to test the hypothesis that DBD is the result of reduced gibberellin synthesis in the fruit during its development on the tree. We will continue with experiments to evaluate the effect of light availability in orchards (with collaboration of FAST Ltd) and determine the influence of Cultar application. In addition it is proposed to evaluate the effects of pre-harvest sprays of gibberellic acid on DBD development in fruit from high-risk orchards.

## **Technology transfer**

An article on the progress of this project appeared in the November 2003 issue of HDC News. Progress reports were presented to growers who attended BIFGA's 16<sup>th</sup> Technical Day held at Bewl Water on 4 February 2004 and the MFFS / EMRA Storage Day held at EMR on 30 March 2004.

## **References**

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