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Grower summary

Headlines

It is not possible to ameliorate the problem of diffuse browning disorder (DBD) by modification of the storage environment.

Early indications that dull weather may enhance DBD development was not proved in shading trials. Cultar (paclobutrazol) application was conducive to DBD in one high-risk orchard.

Some reductions in DBD were achieved by post-harvest application of Berelex^{R} (gibberellic acid or GA_3) and SmartFresh^{R} (1-MCP) but pre-harvest sprays of gibberellic acid were ineffective.

Background and expected deliverables

A hitherto rarely seen storage disorder of Cox apples caused significant losses during the 2000 / 2001 storage season. The disorder is referred to colloquially as Boggy Bank disorder after the first reported occurrence in a Cox orchard of that name. Others refer to the disorder as Gorgate syndrome. First signs are a localised browning of the flesh predominantly towards the calyx end of the fruit (Fig. 1). The disorder progresses around the fruit and may progress to the inner cortex. In the 2000 Cox crop the disorder was first seen in fruit removed from CA storage in early November. There were no reported cases of DBD in air-stored Cox. The disorder often progressed significantly in fruit removed from store and in the worst cases the fruit became unmarketable in a short time.

Figure 1. Diffuse browning disorder (DBD) in Cox apples.

A meeting of advisory and producer groups was held in July 2001 to review the problem and as a result some definitive advice was published (Grower, September 13 2001) on the future prediction and management of the problem. At the time it appeared that the disorder was associated with unusually dull weather conditions in 2000 and it was considered improbable that the disorder would recur to a significant extent for some time. However, contrary to expectations, the disorder occurred to a significant level in some consignments of Cox from the 2001 crop. The occurrence of DBD in the 2001 crop of Cox was significantly less than in the previous year's crop and in many cases rigorous store monitoring and prompt decisions to market affected fruit had minimised commercial losses. Clearly DBD must be regarded as a major threat to the UK fruit industry and an investigation into the cause of the problem has become a priority.

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.

The expected deliverables from the work include:

- An assessment of the potential for controlling the development of DBD through modification of storage conditions and post-storage temperature.
- Assessment of any association between fruit mineral composition and susceptibility to DBD.
- An assessment of the effects of light reduction (by the use of shade netting) and light increase (by the use of reflective mulch) on DBD development.
- An assessment of the effects of the gibberellin inhibitor, paclobutrazol (Cultar) on DBD development.
- An assessment of the effects of exogenous application of gibberellic acid (Berelex^{R}) and 1-MCP (SmartFresh^{R}) on DBD development.

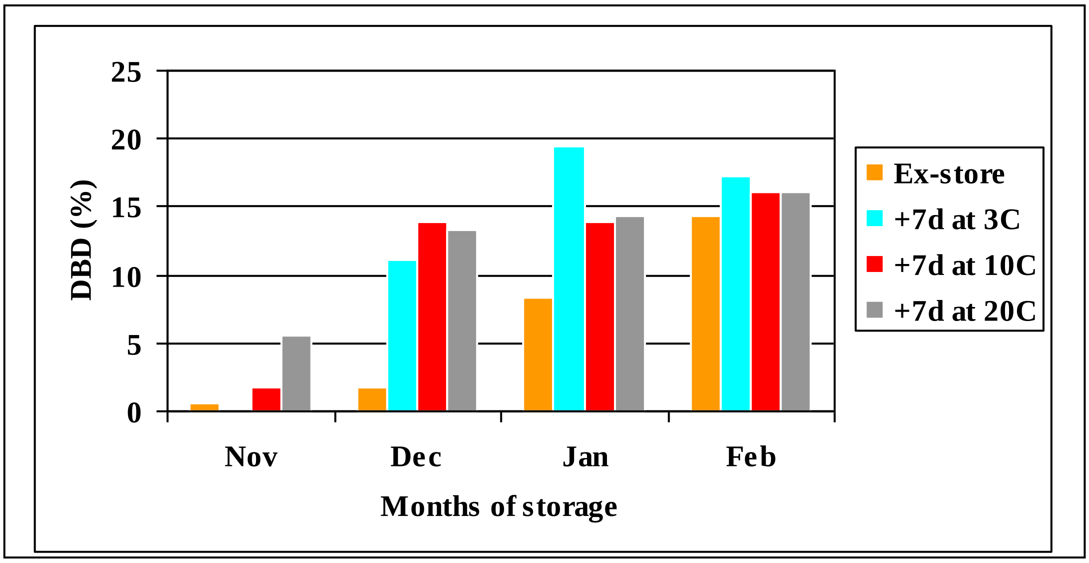
Summary of project and main conclusions

Influence of storage conditions on DBD development

In 2002 samples of fruit were taken during the optimum window for long-term storage from three high-risk orchards in East and West Kent and in Essex. Fruit was stored in three concentrations of oxygen (21% (air), 2% and 1.2% O_2) at two temperatures (1.5-2 and 3.5-4^{o}C). Carbon dioxide concentrations were maintained below 1% using hydrated lime scrubbers. Fruit samples were removed in the middle of November 2002, December 2002, January 2003 and February 2003 and examined immediately and after seven days at 3, 10 and 20^{o}C.

Although DBD was present in a few apples removed from store in November the major increase in its development occurred in fruit removed from store in December and subjected to a further seven days of air storage at 3, 10 or 20^{o}C (Fig. 2). During the 7-day period the average incidence of DBD increased from 2% to 10-15%. This emphasises the importance of ensuring that samples removed from commercial stores for monitoring purposes should be examined after a 7-day period in air in order to detect the first symptoms of DBD. The presence of DBD in any monitoring samples should initiate immediate marketing of the fruit.

Figure 2. DBD development in 2002/2003 - averages for all conditions



DBD developed earlier in the traditional CA regime of 2%O_2 (<1%CO_2) than in ultra-low oxygen conditions of 1.2%O_2 (<1%CO_2). However, by February there was no effect of store oxygen level on the percentage of fruit affected by DBD but CA-stored fruit were more affected than air-stored fruit.

Storage at a temperature (1.5°C) below that recommended (3.5°C) aggravated DBD. It is advisable that a minimum temperature of (4°C) is adopted in commercial CA stores containing fruit from orchards with a history of DBD. The condition of the fruit should be monitored rigorously and the fruit sold early. For short-term air storage the recommended storage temperature of 1.5°C can be used regardless of DBD risk as these fruits should be marketed in October i.e. before DBD is likely to develop in the fruit. The temperature of fruit in CA stores containing fruit from orchards with no history of DBD should be maintained at the correct temperature of 3.5-4°C.

Shelf-life temperature had little effect on the development of DBD. Clearly it is the transfer from CA to air storage that exaggerates the problem, particularly in the early months of storage. At harvest, growers should ensure that they make provision for sufficient numbers of samples for monitoring the quality of fruit in store including a 7-day period in air after removal from CA storage. It is advisable to keep the fruit shelf-life samples at room temperature (18-20°C). Although DBD development was not generally affected by temperature during shelf-life a temperature of 18-20°C is appropriate to monitor changes in other quality attributes such as firmness and the development of other disorders such as bitter pit and breakdown.

The three orchards used in the study had a history of DBD in commercial CA storage. The development of DBD in fruit from these orchards in our experimental stores confirms that the problem is site-related. Overall the two Kent sites in the study developed similar amounts of DBD and were affected more than fruit from the Essex

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site. There was no association between DBD susceptibility and mineral composition of individual apples. Average values of particular mineral nutrients were similar for affected and non-affected fruit. Any possible association of DBD with calcium dependent disorders such as bitter pit can be ruled out in view of the higher average concentration of calcium in affected fruit.

Effect of shading and reflective mulch

A trial was carried out in 2003 and 2004 by FAST Ltd in a commercial Queen Cox orchard with a history of DBD problems. 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 two plots that compared shaded and un-shaded trees. There were four shaded and four 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%.

In 2003 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%.

Over the two years of the trial there was no adverse effect of shading on DBD development but there was a tendency for Extenday to reduce DBD. The lack of any significant effect of shading on DBD development raised doubts about the circumstantial evidence that dull summers are conducive to DBD. The low numbers of reports of DBD in commercial fruit in 2003-4 appeared to support the hypothesis that light level in orchards is a critical factor. The 2003 growing season will be remembered for achieving record temperatures. However, in 2004, a ‘normal’ year as regards sunshine (210 hours) in the period May-August, the occurrences of DBD in commercial fruit exceeded those experienced in the previous worst year (2000) when sunshine hours for the 4-month period averaged only 156 hours.

Effects of Cultar

Cultar (paclobutrazol) is widely used as a means of controlling shoot growth and there had been some speculation that Cultar use is a factor in DBD development. In a trial carried out at EMR in 2003 no DBD was found in CA-stored Cox from trees that had been sprayed with Cultar on six occasions at 0.5L ha^{-1} or on 13 occasions at 0.23L ha^{-1}. However, withholding Cultar in a high-risk orchard in East Kent prevented DBD development in fruit harvested in 2003 and 2004 and reduced DBD incidence in fruit harvested in 2004 from a high-risk orchard in Essex. Although it is clear from commercial experience and from earlier trials done within the project that Cultar use is not the cause of DBD in Cox there is evidence that it is a contributing factor.

Effects of gibberellic acid and 1-MCP (SmartFresh^R) application

In 2004 sprays containing 20 or 200 ppm gibberellic acid (Berelex^R) were applied to trees in Cultar-treated and untreated areas in the commercial orchards with a high-risk of DBD. Sprays were applied on two occasions at the end of August and the beginning of September. Attempts to reduce DBD through the application of gibberellic acid sprays late in the season proved unsuccessful.

Additional samples of fruit were taken in 2004 from the orchards where the effect of Cultar on DBD susceptibility was being investigated. Samples from trees treated or not treated with Cultar were treated with SmartFresh^R (625 ppb for 24 h in a cold room at 3^oC) or immersed for two minutes in a solution containing 400 ppm GA_3. Post-harvest application of gibberellic acid tended to reduce DBD and may warrant further investigation. Similarly the post-harvest application SmartFresh^R (1-MCP) tended to reduce the incidence of DBD. The ability of DBD to develop despite the use of SmartFresh^R to retard ripening suggests that the disorder is incipient at the point of harvest and is not a feature of the normal ripening and senescence process.

Conclusions

Although DBD symptoms are expressed during the storage period it is clear that the potential for the development of the problem originates during fruit development. It is not possible to identify the risk of DBD in particular consignments of fruit at the point of harvest. DBD can occur in fruit that is harvested at the correct stage of maturity for long-term storage and with a mineral composition that meets the required standards. Consequently it is imperative that the quality of Cox in commercial stores is monitored rigorously and regularly. The disorder develops rapidly once fruit is removed from CA conditions. It is imperative that monitoring of fruit quality is done immediately ex-store and again after seven days at room temperature. Detection of DBD in samples at any stage should initiate immediate marketing of the fruit. There is little that can be done to ameliorate DBD in susceptible consignments by modification of storage practice.

It appears that fruits that are susceptible to DBD in CA storage have a high susceptibility to other forms of flesh breakdown in air storage such as low temperature and senescent breakdown. The pre-harvest factors that are responsible for initiating stress during fruit development that result in DBD development during storage are unresolved. Given the rapid ‘spread’ of the problem in the 2004 crop it is likely that short-term changes in management practice are the cause of the problem. There was speculation that unusual weather conditions were responsible for seasonal variation in susceptibility and that dull, sunless summers may be conducive to the problem. However this association was contradicted in 2004, which proved the worst year for DBD despite normal hours of sunshine during fruit development. The results of two years of shading trials also indicated no effect of light reduction on susceptibility of fruit to DBD.

The cause of DBD remains obscure and no specific advice can be provided to the fruit industry as regards possible cause and measures to reduce the risk of DBD. Clearly the use of agrochemicals in orchards needs to be considered as a potential cause of DBD and further work is required to examine the use of specific pesticides and to document dose, timing and combinations of materials. It is known that many pesticides invoke physiological and biochemical changes that are not associated with target response of the treatment. The use of Cultar (paclobutrazol) appears to be a factor in DBD development but is clearly not the sole cause of the problem. At one high-risk site in East Kent the DBD problem was alleviated in two years by avoiding the use of Cultar. However, in an orchard with no history of DBD, it was not possible to induce the disorder even where 13 sprays of Cultar were applied from early June to early September.

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 program are the cause of the stress 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.

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. This was particularly evident in the 2004 storage season. 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 was helpful in providing advice on how to manage crops of fruit from orchards with a history of DBD. These action points are given below.

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^{o}C and marketed early.
- Growers can use either 1.2 or 2%O_2 (<1%CO_2) for the storage of Cox regardless of DBD risk. It is not known whether storage in 5%CO_2 + 3%O_2 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^{o}C) for CA storage.
- Regardless of DBD risk fruit stored in air should be kept at 1.5^{o}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 seven days at room temperature (18-20^{o}C).
- Detection of DBD should prompt immediate marketing.
- In orchards with a history of DBD growers should withhold Cultar from designated areas. Bins of fruit from different areas should be identified and monitored separately.
- In orchards with a history of DBD growers are advised to designate areas where nil or minimal pesticides are used. Careful monitoring of fruit during storage should help to determine the contribution of the spray programme to the DBD problem.

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Science Section

Introduction

A hitherto rarely seen storage disorder of Cox apples caused significant losses during the 2000/2001 storage season. The disorder is referred to colloquially as Boggy Bank disorder after the first reported occurrence in a Cox orchard of that name. Others refer to the disorder as Gorgate syndrome. First signs are a localised browning of the flesh predominantly towards the calyx end of the fruit. The disorder progresses around the fruit and may progress to the inner cortex. In the 2000 Cox crop the disorder was first seen in fruit removed from CA storage in early November. There were no reported cases of diffuse browning disorder (DBD) in air-stored Cox. The disorder often progressed significantly in fruit removed from store and in the worst cases the fruit became unmarketable in a short time.

A meeting of advisory and producer groups was held in July 2001 to review the problem and as a result some definitive advice was published (Grower, September 13 2001) on the future prediction and management of the problem. At the time it appeared that the disorder was associated with unusually dull weather conditions in 2000 and it was considered improbable that the disorder would recur to a significant level for some time. However, contrary to expectations, the disorder occurred to a significant level in some consignments of Cox from the 2001 crop. The occurrence of DBD in the 2001 crop of Cox was significantly less than in the previous year's crop and in many cases rigorous store monitoring and prompt decisions to market affected consignments of fruit had minimised commercial losses.

Aims of the project

The overall aim of the project is to identify the factors that induce diffuse browning disorder (DBD) in Cox apples and to suggest measures that will prevent development of the disorder in CA-stored fruit.

Outline of results from year 1

The following is a summary of the results obtained in the first year of the trial. A more detailed account of the work carried out in year one can be found in the First Year Report to HDC (Johnson, 2003).

Time course for DBD development

Although DBD was present in a few apples removed from store in November the major increase in its development occurred in fruit removed from store in December and subjected to a further seven days of air storage at 3, 10 or 20°C. During the 7-day period the average incidence of DBD increased from 2% to 10-15%. This emphasises the importance of ensuring that samples removed from commercial stores for monitoring purposes should be examined after a 7-day period in air in order to detect the first symptoms of DBD. The presence of DBD in any monitoring samples should initiate immediate marketing of the fruit.

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Influence of store oxygen concentration on the development of DBD

DBD developed earlier in the traditional CA regime of 2%O_2 (<1%CO_2) than in ultra-low oxygen conditions of 1.2%O_2 (<1%CO_2). However, by February there was no effect of store oxygen level on the percentage of fruit affected by DBD but CA-stored fruit were more affected than air-stored fruit.

Influence of store temperature on the development of DBD

Storage at a temperature (1.5°C) below that recommended (3.5°C) aggravated DBD. It is advisable that a minimum temperature of (4°C) is adopted for commercial CA stores containing fruit from orchards with a history of DBD. The condition of the fruit should be monitored rigorously and the fruit sold early. For short-term air storage the recommended storage temperature of 1.5°C can be used regardless of DBD risk as these fruits should be marketed in October i.e. before DBD is likely to develop in the fruit. The temperature of fruit in CA stores containing fruit from orchards with no history of DBD should be maintained at the correct temperature of 3.5-4°C.

Influence of shelf-life temperature on the development of DBD

Shelf-life temperature had little effect on the development of DBD. Clearly it is the transfer from CA to air storage that exaggerates the problem, particularly in the early months of storage. At harvest growers should ensure that they make provision for sufficient numbers of samples for monitoring the quality of fruit in store including a 7-day period in air after removal from CA storage. It is advisable to keep the shelf-life samples at room temperature (18-20°C). Although DBD development was not generally affected by temperature during shelf-life a temperature of 18-20°C is appropriate to monitor changes in other quality attributes such as firmness and the development of other disorders such as bitter pit and breakdown.

Variation in the incidence of DBD due to orchard site

The three orchards used in the study had a history of DBD in commercial CA storage. The development of DBD in fruit from these orchards in our experimental stores confirms that the problem is site-related. Overall the two Kent sites in the study developed similar amounts of DBD and were affected more than fruit from the Essex site.

Outline of results from year 2

The following is a summary of the results obtained in the second year of the trial. A more detailed account of the work carried out in year two can be found in the Second Year Report to HDC (Johnson, 2004).

Effects of Cultar

Application of Cultar in commercial orchards had no affect 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

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greater in fruit from the Essex orchard where the firmness benefit was commercially significant. DBD developed in fruit from the East Kent orchard but 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 where Cultar was applied on six or 13 occasions at 0.5 and 0.23 L ha^{-1} respectively 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 reflective mulch

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 plots 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 2004 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 or definite category for DBD 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.

Targets for Year 3

In the final year of the project research continued to test the hypothesis that DBD is the result of reduced gibberellin synthesis in the fruit during its development on the tree. Experiments to evaluate the effect of light availability in orchards were continued (with collaboration of FAST Ltd) and experiments were carried out to determine the influence of Cultar application on DBD development. Additionally it was proposed to evaluate the effect of applying gibberellic acid directly to the fruits either as late season sprays or as a post-harvest dipping treatment. The effect of SmartFresh^{R} on DBD development was also investigated using fruit from high-risk orchards.

Materials and Methods

Cultar application in commercial orchards

Two orchards with high risk of DBD that were used in the previous year were selected for further evaluation of the effect of Cultar on the development of the disorder. In the East Kent orchard (ref. TC) the three rows (approximately 150 trees) that were not treated with Cultar in 2003 were again left untreated. In the Essex orchard (ref. FH) the area that was not treated with Cultar in 2004 was increased significantly from that left unsprayed in 2003. An entire block of trees planted in a 4-row bed system received no Cultar sprays whereas the remaining blocks (single-row system) in the orchard received a normal Cultar programme. Samples of fruit for storage were taken on 6 (TC) and 13 (FH) September 2004 and transported immediately to the Jim Mount Building at East Malling. In order to obtain representative samples from the treated and untreated areas two fruits were sampled at waist-height from about 100 trees in the treated rows and trees in the Cultar-treated areas were sampled in the same way. On arrival at East Malling the fruit from the Cultar-treated and untreated areas were randomised to form samples for storage in CA (1.2% O_2, <1% CO_2) at 3.5-4^{o}C. Further samples were taken for mineral analysis by a commercial analytical laboratory (NRM Ltd).

Gibberellic acid application in commercial orchards

Sprays containing gibberellic acid or GA_3 (Berelex^{R}) were applied to trees in the Cultar-treated and untreated areas in the commercial orchards with a high-risk of DBD (see above). In each area there were three treatments (untreated, 20 ppm and 200 ppm GA_3) replicated six times with single-tree plots. Sprays were applied to run-off on two occasions (TC – 23 August and 1 September, FH – 25 August and 1

September 2004) using a back-pack sprayer. Fruit was harvested at the same time as the Cultar trials (see above). Samples of fruit were stored in air at low temperature (0-0.5^{o}) to induce low temperature breakdown (LTB) and in recommended CA conditions (1.2% O_2, <1% CO_2) at 3.5-4^{o}C. Samples were removed from air and CA storage on 2, 7 and 22 February 2005 respectively and assessed for internal disorders.

Post-harvest application of gibberellic acid or SmartFresh^{R}

Additional samples of fruit were taken from the orchards where the effect of Cultar on DBD susceptibility was being investigated. Samples treated or not treated with Cultar were treated with SmartFresh^{R} (625 ppb for 24 h in a cold room at 3^{o}C) or immersed for 2 minutes in a solution containing 400 ppm GA_3 (TC orchard only). Samples were stored in CA conditions (1.2% O_2, <1% CO_2) at 3.5-4^{o}C until 14 February. After removal the samples were weighed and fruits were examined for the presence of external disorders and rots. Ten 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 (see below). A second set of samples was kept for a further seven days at 20^{o}C and then examined for rots and internal defects.

Shading / Extenday trial in a commercial orchard

The trial carried out in 2003 by FAST Ltd in a commercial orchard of Queen Cox situated in North Kent (Lynsted) was repeated in 2004. 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. Over 20% of the fruit sampled from the experimental area in 2003 developed DBD-like symptoms during CA storage. 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 two plots that compared shaded and un-shaded trees. There were four shaded and four 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 experimental trees were harvested on 21 September 2004 and sub-samples for storage were transported immediately to the post harvest facility (Jim Mount Building) at East Malling Research. Fruit was removed from CA storage (1.2% O_2, <1% CO_2 at 3.5-4^{o}C) on 14 February 2005 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 (see below). A second set of samples was kept for a further seven days at 20^{o}C and then examined for internal defects.

<u>Red colour.</u> The percentage area of red colour on each apple was estimated and assigned to one of six categories i.e. 0, 1-20, 21-40, 41-60, 61-80 and >80% that were ascribed a score of 0, 1, 2, 3, 4 and 5 respectively. The maximum score for red colour in a 20-fruit sample was 100. There was no attempt to assess the intensity of red colour.

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.

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.

Internal disorders. Each fruit was cut at the calyx end and at the equator and examined for the presence of disorders. The incidence of each type of disorder was recorded.

Statistical analysis

Data from the various trials were 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 but original means are provided in the tables of results.

Results and Discussion

Cultar application in commercial orchards

As in 2003, fruits from trees treated with Cultar tended to be higher in potassium and phosphorus but the levels of both nutrients were below those considered optimum for storage and the low levels of phosphorus were of particular concern as regards increased risk of low temperature breakdown (Table 1). Fruit from the Cultar-treated area in the Essex orchard (FH) was lower in nitrogen but this may be associated more with the type of planting (4-row compared with single row) than application of Cultar. Higher levels of P and K levels in fruit are often associated with lighter cropping. No comment can be made on this aspect as no attempt was made to estimate the yield of trees in Cultar-treated and untreated areas.

There were two sources of information on the effects of Cultar on storage quality. The first was from bulked samples that were also used to investigate the effects of post-harvest application of SmartFresh^R and GA_3 (Table 7) and the second was from the experiments investigating the effect of gibberellin sprays (Tables 3 and 4).

In bulked samples from orchard TC 17% of the fruit from Cultar-treated trees developed DBD in CA storage compared with 0% in fruit from trees that received no Cultar (Table 7). There was insufficient incidence of DBD in fruit from the Essex orchard (FH) to determine any effect of Cultar. There were no effects of Cultar on firmness or background colour of fruits from the East Kent orchard (TC) but Cultar-treated fruit from the Essex orchard (FH) were firmer and less green than those from areas not treated with Cultar. These effects may be attributed more to the different orchard systems than to a direct effect of 'Cultar'. However, the firmness benefit from

Cultar use is consistent with the results obtained in the previous year where there was no difference in orchard system i.e. all single rows.

The control fruit from the gibberellin spray experiments showed significant effects of Cultar in promoting the development of DBD symptoms in the fruit (Table 3). Cultar-treated fruit from the East Kent orchard did not develop such a high level of DBD as the bulked samples. This is probably explained by the lower number of trees (six) sampled per treatment in the gibberellin spray trial compared with the bulked samples (approximately 100). There were no effects of Cultar on the incidence of rotting or other disorders.

As expected, fruit stored in air at 0-0.5°C until February 2005 was severely affected by low temperature breakdown (LTB). The incidence of LTB in fruit from the Cultar-treated area in the East Kent orchard was twice that in fruit from the untreated area (Table 4). Susceptibility of CA-stored fruit to DBD was therefore associated with a heightened susceptibility to LTB in the East Kent fruit. However, in the Essex fruit where DBD levels were slight and effects of Cultar were less marked there was no significant effect of Cultar on LTB development. It was noted previously that susceptibility to DBD was associated with higher susceptibility to senescent and low temperature breakdown in air storage. It therefore appears that DBD is an expression of a general stress that exists in the fruit at the time of harvest and that CA favours expression in the form of DBD whereas in air storage the symptoms are more likely to develop as recognised forms of flesh breakdown.

Table 1. Analysis of major minerals (mg $100g^{-1}$) in bulk samples of fruit at harvest in 2004 and standards for Cox stored in CA conditions of 1.2% O_2, <1% CO_2 at 3.5-4°C.

Site	Cultar	Mean fruit wt (g)	N	P	K	Ca	Mg	K/Ca
TC	No	3230	63	8.1	93	5.1	4.7	18.2
	Yes	3401	69	8.7	105	5.2	5.3	20.5
FH	No	3371	55	8.4	111	4.4	4.8	25.2
	Yes	3333	34	10.0	124	4.4	4.9	28.3
Standards for CA			50-70	11 min	130-150	4.5	5	35

Table 2. Analysis of minor and trace minerals (mg $100g^{-1}$) in bulk samples of fruit at harvest in 2004.

Site	Cultar	B	Cu	Mn	Zn
TC	No	0.40	0.048	0.062	0.080
	Yes	0.22	0.036	0.058	0.019
FH	No	0.35	0.023	0.046	0.013
	Yes	0.39	0.023	0.040	0.017

Table 3. The effect of Cultar sprays on the incidence (%) of rotting and storage disorders in Cox apples from an East Kent (TC) and Essex (FH) orchard stored in CA (<1% CO_2 + 1.2% O_2) at 3.5ºC until late February 2005.

Orchard	Sprays	Rots (%)	Scald (%)	Bitter pit (%)	DBD (%)	Core flush (%)	Break-down (%)
TC	Yes	7.8	15.9	3.5	4.9	0.4	0.2
	No	4.0	13.4	4.2	0.0	0.9	0.0
	SED (30 d.f.)	2.30	3.57	2.35	1.10	-	-
FH	Yes	2.7	0	11.9	3.0	0.2	2.3
	No	5.3	0	18.8	0.2	0.0	2.0
	SED (30 d.f.)	1.36	-	5.06	1.21	-	1.66

Table 4. The effect of Cultar sprays on the incidence (%) and severity of breakdown in Cox apples from an East Kent (TC) and Essex (FH) orchard stored in air at 0-0.5ºC until early February 2005.

Orchard	Cultar	Breakdown		
		External	Internal	
		%	%	Severity (max 60)
TC	Yes	40.1	67.3	19.9
	No	20.6	47.4	16.2
	SED (30 d.f.)	4.35	6.88	2.85
FH	Yes	22.7	41.7	14.6
	No	25.2	46.9	15.2
	SED (30 d.f.)	5.18	7.31	3.09

Gibberellic acid application in commercial orchards

The incidence of DBD in CA-stored fruit was generally too low to show any potential effects of GA_3 sprays although in fruit from both orchards unsprayed fruit tended to be least affected (Table 5). There were no significant effects of GA_3 sprays on the levels on rotting or other disorders in the fruit. There was a tendency for GA_3 sprays to increase the incidence of LTB in fruit stored in air at 0-0.5ºC which is contrary to published data obtained in Australia (Wills et al., 1973).

Table 5. The effect of gibberellic acid (GA_3) sprays on the incidence (%) of rotting and storage disorders in Cox apples from an East Kent (TC) and Essex (FH) orchard stored in CA (<1% CO_2 + 1.2% O_2) at 3.5ºC until late February 2005.

Orchard	Sprays	Rots (%)	Scald (%)	Bitter pit (%)	DBD* (%)	Core flush (%)	Break-down (%)
TC	GA_3 20 ppm	6.7	15.2	3.4	2.4	0.0	0.0
	GA_3 200 ppm	5.3	10.3	3.3	3.5	1.3	0.3
	Control	5.7	18.4	4.9	1.4	0.7	0.0
	SED (30 d.f.)	2.82	4.37	2.87	1.35	0.73	-
FH	GA_3 20 ppm	5.7	0	9.5	2.8	0	2.4
	GA_3 200 ppm	3.7	0	22.0	1.7	0	3.3
	Control	2.7	0	14.6	0.4	0.4	0.7
	SED (30 d.f.)	1.66	-	6.19	1.49	-	2.04

- Insufficient data for statistical analysis

Table 6. The effect of gibberellic acid (GA_3) sprays on the incidence (%) and severity of breakdown in Cox apples from an East Kent (TC) and Essex (FH) orchard stored in air at 0-0.5ºC until early February 2005.

Orchard	Sprays	Breakdown		
		External	Internal	
		%	%	Severity (max 60)
TC	GA_3 20 ppm	37.5	61.6	20.9
	GA_3 200 ppm	23.8	54.3	15.7
	Control	29.7	56.2	17.5
	SED (30 d.f.)	5.33	8.43	3.49
FH	GA_3 20 ppm	25.2	46.8	16.3
	GA_3 200 ppm	25.6	48.0	16.3
	Control	21.1	38.1	12.1
	SED (30 d.f.)	6.35	8.96	3.79

Post-harvest application of gibberellic acid or SmartFresh^R

As expected the application of SmartFresh^R improved the firmness of CA-stored fruit from both the East Kent (TC) and Essex (FH) orchards and tended to reduce the incidence of DBD in the East Kent fruit although the effect just failed to reach statistical significance at the 5% level of probability (Table 7). There was insufficient DBD in the Essex fruit to test the effect of SmartFresh^R. Fifty percent of fruit from the Essex orchard that was treated with SmartFresh^R developed russet stain whereas the Kent fruit were unaffected. Applying gibberellic acid post-harvest increased the firmness and tended to reduce the incidence of DBD in CA-stored Cox apples from the East Kent orchard. There was insufficient GA_3 available to treat the fruit from the Essex orchard.

Table 7. The effects of post harvest application of SmartFresh or gibberellic acid (GA) on the storage quality of Queen 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 CA (<1% CO_2 + 1.2% O_2) at 3.5ºC until 15 February 2005.

Site	Cultar	Smart -Fresh	GA dip	Russet stain (%)	Ground colour (1, green-4, yellow)	Firmness (N)	DBD (%)
TC	No			0	1.34	65.1	0.0
	Yes			0	1.33	65.7	17.0
		No		0	1.31	55.5	11.0
		Yes		0	1.34	75.2	6.0
			No	0	1.31	55.5	11.0
			Yes	0	1.45	62.7	7.0
SED				-	0.055	1.62	2.58
FH	No			25.0	1.94	65.8	1.0
	Yes			25.0	2.21	77.1	2.0
		No		0.0	2.03	61.1	2.0
		Yes		50.0	2.12	81.9	1.0
SED				5.0	0.068	1.16	-

Shading / Extenday trial in a commercial orchard

Shading reduced the mean weight and mean diameter of fruit but did not affect red colour development or grade-out in terms of percentage Class 1 fruit (Table 11). Extenday had no effects on fruit size, red colour or grade-out.

Table 8. The effects of shading and Extenday treatments on the grade-out of Queen Cox apples at harvest in 2004.

	Extenday		s.e.d.	Shade		s.e.d.
	Yes	No	(2 df)	No	Yes	(6 df)
Mean weight (g)	196.7	165.9	9.39	194.1	168.5	9.73
Mean diameter (mm)	76.4	71.4	2.72	75.8	71.9	1.36
Red colour (%)	48.5	35.6	6.19	43.9	40.2	9.96
Class 1 (%)	76.1	62.2	10.67	70.6	67.7	10.41

The quality of fruits removed from CA storage on 21 February 2005 was generally not significantly affected by shading or by Extenday (Table 12). However, shaded fruits tended to be smaller and less red and had a significantly greener background colour. These results agree with those obtained in the previous year where treatment effects were generally more significant statistically. As found in the previous year (2003 crop), the quality of fruits removed from CA storage was not affected by Extenday.

Internal examination of the fruit revealed a range of browning disorders that may or may not be related (Table 13). On average 13.1% of fruits were affected by classical symptoms of DBD but additionally in some fruits DBD was insufficiently defined. In some fruit there was a general browning in the calyx region of the fruit and this was scored separately from DBD but also combined to give a total of fruit affected by all DBD-like symptoms. As in the previous year there was no adverse effect of shading on DBD development but there was a tendency for Extenday to reduce DBD and there had been a slight beneficial effects of Extenday in the previous year.

Contrary to the previous year circumstantial evidence that DBD is encouraged by low light conditions was not supported by the high incidence of the problem in commercial fruit produced in the ‘normal light’ year of 2004. Moreover, shading trees to reduce light availability did not exacerbate DBD and there was no significant ameliorating effect of Extenday.

Over the last two years the experimental results have not supported 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 again confirms an orchard factor influence in the problem.

Table 9. The effects of shading and Extenday treatments on the size and quality of Queen Cox apples stored in 1.2% O_2 + <1% CO_2 at 3.5°C until 21 February 2005.

	Mean fruit weight (g)	**Ground colour (1= green, 4= yellow)**	**Red colour (5 = 100% cover)**	**Firmness (N)**
Shade	176.5	1.94	3.49	49.0
No shade	202.4	2.35	3.83	48.2
s.e.d (6 d.f.)	11.15	0.142	0.306	3.12
Extenday	204.6	2.04	3.55	49.3
No Extenday	174.3	2.25	3.76	47.9
s.e.d (2 d.f.)	22.51	0.325	0.151	3.31

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Table 10. The effects of shading and Extenday treatments on the incidence (%) of calyx browning and diffuse browning disorder (DBD) in Queen Cox apples stored in 1.2% O_2 + <1% CO_2 at 3.5°C until 21 February 2005. Percentage data were converted to angles prior to analysis.

	Ex-store	Ex - 7 days at 20°C			Average ex-store and ex - 7 days at 20°C
	DBD (%)	DBD (%)	Calyx browning (%)	All DBD-like symptoms (%)	DBD (%)
Shade	11.1	8.9	4.0	12.9	10.0
No shade	15.2	8.8	1.3	10.0	12.0
s.e.d (6 d.f.)	7.09	6.27	1.11	6.16	6.24
Extenday	12.7	5.1	2.6	7.8	8.9
No Extenday	13.6	12.5	2.6	15.1	13.1
s.e.d (2 d.f.)	6.72	2.93	2.88	5.82	4.82

Conclusions

It is clear from the work done in the first year of the project that susceptibility to DBD is determined during fruit development and that little can be done to ameliorate the problem by modifying storage parameters. DBD symptoms were first evident in CA-stored fruit during November and the incidence of affected fruit increased with storage time until mid February. It appears that the potential incidence of DBD is already determined at the point of harvest but there is no way of knowing at that time what the potential problem is. DBD was aggravated by lower storage temperatures but development of the disorder was similar in fruit stored in the traditional CA regime of 2%O_2 (<1%CO_2) and in ultra-low oxygen conditions of 1.2%O_2 (<1%CO_2). It is advised that the temperature of fruit in CA stores containing fruit from orchards with no history of DBD should be maintained at the correct temperature of 3.5-4°C. However, it is advisable that a minimum temperature of (4°C) is adopted for fruit in commercial CA stores containing fruit from orchards with a history of DBD.

The number of fruit affected by DBD and the severity of the symptoms increases markedly once fruit are removed from CA conditions but the temperature at which fruit is held after storage had little effect on DBD development. Rigorous monitoring of fruit during storage is crucial in order to avoid loss of consignments due to DBD. Samples removed from commercial stores for monitoring purposes should be examined after a 7-day period in air in order to detect the first symptoms of DBD. The presence of DBD in any monitoring samples should initiate immediate marketing of the fruit. It is advisable to keep the shelf-life samples at room temperature (18-20°C). Although DBD development was not generally affected by temperature during shelf-life a temperature of 18-20°C is appropriate to monitor changes in other quality attributes such as firmness and the development of other disorders such as bitter pit and breakdown.

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 DBD problem. This was based on circumstantial evidence that DBD was worse in dull growing seasons and the potential involvement of the gibberellin inhibitor Cultar for growth control. The low numbers of reports of DBD in commercial fruit in 2003-4 appeared to support the hypothesis that light level in orchards is a critical factor. The 2003 growing season will be remembered for achieving record temperatures. In 2004 hours of sunshine calculated at EMR for the period May-August inclusive was 210, which compares well with the long-term average of 206 hours for this period. Despite a low predicted incidence of DBD in the 2004 crop the actual occurrences exceeded those experienced in the previous worst year (2000) when sunshine hours for the 4-month period averaged only 156 hours.

A link between light availability and DBD susceptibility was not established in the shading trials carried out by FAST Ltd and there were no marked effects on the incidence of DBD of providing additional light into the trees by the use of reflective mulch.

Although it is clear from commercial experience and from earlier trials done within the project that Cultar use is not the cause of DBD in Cox there is evidence that it is a contributing factor. Withholding Cultar in a high-risk orchard in East Kent prevented DBD development in fruit harvested in 2003 and 2004 and reduced DBD incidence in fruit harvested in 2004 from a high-risk orchard in Essex. Attempts to reduce DBD through the application of gibberellic acid sprays late in the season were unsuccessful. However, the extent to which endogenous gibberellin levels were affected by spray application is not known. Application of gibberellic acid as a post-harvest dipping treatment tended to reduce DBD and may warrant further investigation. Similarly, the post-harvest application SmartFresh^{R} tended to reduce the incidence of DBD. The ability of DBD to develop despite the use of SmartFresh^{R} to retard ripening suggests that the disorder is incipient at the point of harvest and is not a feature of the normal ripening and senescence process. It is possible that the application of certain types of agrochemicals (or combinations) at particular stages of fruit development are initiating stresses in the fruit that eventually results in cell death and DBD development at some stage in the storage period. Such dramatic effects of chemical sprays on storage quality of Cox are not unprecedented. Perhaps the best example was the effect of daminozide (Alar or B-nine), which induced a particular type of breakdown in stored Cox apples and seriously aggravated recognised disorders such as core flush. Clearly it is of paramount importance to prove conclusively that agrochemical sprays are the cause of the DBD problem before further work can be proposed to identify specific factors and to suggest remedial action.

Technology transfer

An article on the progress of this project appeared in the November 2003 issue of HDC News.

Progress report to growers who attended BIFGA's 16th Technical Day held at Bewl Water, Lamberhurst on 4 February 2004.

Progress report to growers who attended the MFFS / EMRA Storage Day held at EMR on 30 March 2004.

Progress report to growers who attended the MFFS / EMRA Storage Day held at EMR on 5 April 2005.

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