

**Project Title:** Influence of agrochemical use on the development of diffuse browning disorder (DBD) in Cox's Orange Pippin apples

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## **Grower summary**

### **Headlines**

Grower trials targeting the bloom period to compare different fungicide (and growth regulator) sprays did not produce consistent effects on the development of diffuse browning disorder.

### **Background and expected deliverables**

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

The HDC funded a three-year project (TF 139) to investigate the cause of DBD and, although much useful information was gained on factors that may influence the development of DBD during storage, the predisposing factors have not been identified (Johnson, 2005, 2006a and 2006b).

DBD remains a major threat to the UK fruit industry and has affected the confidence of growers to store Cox for more than short-term. Further work is required to confirm the suspicion that factors in the pesticide spray programme may be 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. The specific aim of the project was to examine the possible relationship between DBD and the application and timing of specific types of agrochemicals.

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

Eight farms were visited by EMR staff in late August 2005 to view orchard trials set up by the growers, mainly in collaboration with staff from Hutchinson Ltd. These farms were visited again during the harvesting period and samples of fruit were taken from relevant areas within each of the orchards. Two replicate samples comprised of 50 fruit were taken from each plot. One apple was selected at random from each of 50 trees and placed into a sample net and labelled. The procedure was repeated to form a duplicate sample. A total of 37 plots were sampled (74 samples) during the optimum harvesting period for Cox and taken to EMR for storage. Samples were placed into bulk storage bins and loaded into new 20-tonne stores. The fruit was cooled to 3.5°C and the stores were sealed. The stores were slow to achieve the target oxygen concentration of 1.2% and for most of the storage period an injection of nitrogen gas was required to maintain conditions. Carbon dioxide was maintained at very low concentrations (0.1%) by hydrated lime placed directly in the store with the fruit. The stores were opened on 6 February 2006 and the sample nets were removed from the bins and placed in a room at 15°C for 7 days. Sample nets were weighed and the number of rotten fruit was counted. Apples seriously affected by fungal decay were discarded and the remaining apples were cut at the calyx end and at the equator and examined for the presence of physiological disorders. The incidence of each type of disorder was recorded. The number and weight of affected fruit in a sample was recorded and this affected fruit was reserved for mineral analysis. A similar number and weight of healthy fruit was selected from the sample and reserved for mineral analysis. Forty-five samples were sent to NRM Laboratories for analysis of N, P, K, Ca, Mg, B, Cu, Mn, Fe and Zn.

The incidence of diffuse browning disorder in fruit from the participating farms varied from 0 to 23% (average 7.2%). It was interesting that most of the orchards were considered to be high-risk based on occurrence of the problem in previous years and yet only two of the orchards were affected significantly in 2005. The fact that

orchards can change their susceptibility status in different growing seasons affords the prospect of controlling the problem once the underlying cause of DBD is understood.

Results showed the following:

- Targeting the bloom period to compare different fungicide (and growth regulator) sprays in 2005 didn't produce consistent effects
- Over sprays of 'Systhane' and / or 'Cultar' applied in early May did not affect DBD incidence compared with a standard programme of 'Captan', 'Scala', 'Nimrod' and 'Regalis'
- Fruit from orchards that received bloom sprays containing 'Systhane' and 'Stroby' were affected by DBD to a similar extent to those sprayed with 'Captan', 'Scala' and 'Nimrod'
- At many sites there was insufficient DBD to make comparisons of different spray treatments

In the 2002 crop there was no association between DBD susceptibility and mineral composition of individual apples (see final report on HDC project TF 139). A similar exercise was carried out in 2005 with bulked samples of affected and unaffected fruit from twelve commercial orchards/plots. Again, DBD was not related to mineral composition of the fruit at harvest. It remains possible that DBD could be related to a trace nutrient that has not been considered or to a transient shortage of a particular nutrient(s) during fruit development. These possibilities have not been tested.

## **Conclusions**

The actual cause of DBD remains obscure and there are no orchard factors that consistently affect susceptibility. The problem is reminiscent of that of bitter pit before an association with calcium nutrition was established, with many factors being influential but none consistently so. Once it was clear that bitter pit was only likely in

fruit that did not achieve a sufficient threshold level of calcium then the impact of orchard factors could be interpreted.

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

In the longer term, a larger-scale research project is envisaged to tackle this complex problem, possibly in the form of a HortLINK project sponsored by Defra, HDC and the UK top fruit industry.

### **Financial benefits**

Growers with orchards that are known to be at risk are restricted to storing fruit short-term. In some years, this may result in significant financial loss due to the necessity to market at a time when the markets are traditionally over-supplied with dessert apples. More importantly there is a lack of confidence in storing Cox due to the threat of DBD, even where problems have not arisen in the past. It is difficult to quantify the financial implications of forced changes in the marketing strategy for UK Cox. It is easier to cost the loss of consignments of fruit rejected due to the presence of DBD. There are cases of complete losses of stores where retail value of 100 tonnes is in the region of £100,000. The work done previously in HDC project TF 139 was helpful in providing advice on how to manage crops of fruit from orchards with a history of DBD. In view of the inconclusive nature of the results obtained in this one-year project no additional advice can be provided on avoidance of the problem. Consequently there is no direct financial benefit of the work.

### **Action points for growers**

- Growers, with the help of their advisers, should adopt a systematic approach to assess the potential impact of agrochemicals on DBD susceptibility.

- Dividing orchards, and replicating treatments where possible, is preferable to spraying different orchards with different regimes.
- Identifying bins at picking and through storage and grading could prove particularly helpful in assessing the possible contribution of agrochemicals to DBD development.

## **Science Section**

### **Introduction**

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

The HDC funded a three-year project (TF 139) to investigate the cause of DBD and although much useful information was gained on factors that may influence the development of DBD during storage the predisposing factors have not been identified (Johnson, 2005, 2006a and 2006b)

DBD is a major threat to the UK fruit industry and has affected the confidence of growers to store Cox for more than short-term. Further work is required to confirm the suspicion that factors in the pesticide spray programme 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.

### **Aims of the project**

To examine the possible relationship between DBD and the application and timing of specific types of agrochemicals



## **Materials and Methods**

Eight farms were visited by EMR staff in late August 2005 to view orchard trials set up by the growers mainly in collaboration with staff from Hutchinson Ltd. These farms were visited again during the harvesting period and samples of fruit were taken from relevant areas within each of the orchards. Two replicate samples comprised of 50 fruit were taken from each plot. One apple was selected at random from each of 50 trees and placed into a sample net and labelled. The procedure was repeated to form a duplicate sample. A total of 37 plots were sampled (74 samples) during the optimum harvesting period for Cox and taken to EMR for storage. Samples were placed into bulk storage bins and loaded into new 20-tonne stores. The fruit was cooled to 3.5°C and the stores were sealed. The stores were slow to achieve the target oxygen concentration of 1.2% and for most of the storage period an injection of nitrogen gas was required to maintain conditions. Carbon dioxide was maintained at very low concentrations (0.1%) by hydrated lime placed directly in the store with the fruit. The stores were opened on 6 February 2006 and the sample nets were removed from the bins and placed in a room at 15°C for 7 days. Sample nets were weighed and the number of rotten fruit was counted. Apples seriously affected by fungal decay were discarded and the remaining apples were cut at the calyx end and at the equator and examined for the presence of physiological disorders. The incidence of each type of disorder was recorded. The number and weight of affected fruit in a sample was recorded and this affected fruit was reserved for mineral analysis. A similar number and weight of healthy fruit was selected from the sample and reserved for mineral analysis. Forty-five samples were sent to NRM Laboratories for analysis of N, P, K, Ca, Mg, B, Cu, Mn, Fe and Zn.

### *Statistical analysis*

In most cases, a formal statistical analysis of the data was not possible due to lack of any replication of the treatments in the orchards. It was possible to analyse some data sets using an analysis of variance (ANOVA) and correlation / regression analysis with GENSTAT 7 statistical software.

## Results and Discussion

### *Overall level of DBD in fruit from orchards samples in 2005*

The average percentage of fruit affected by classic symptoms of DBD was only 4.6%, although the incidence of DBD in fruit from the treated plots varied from 0 to 32%. Classic DBD in fruit from the various farms varied from 0 to 16%. Some fruits were affected by a general browning at the tip of the calyx end and this symptom was recorded separately, although it was undoubtedly an early symptom of DBD. The average percentage of fruit affected by either type of diffuse browning was 7.2% and varied from 0 to 44%. The incidence of either type of disorder in fruit from the participating farms varied from 0 to 23% (Table 1). It was interesting that most of the orchards were considered to be high-risk based on occurrence of the problem in previous years and yet only two of the orchards were affected significantly in 2005. Orchard reference S had no history of DBD and again proved to be non-susceptible to the problem. It is interesting to note that orchard S is located adjacent to the BL orchards which were badly affected in 2004. The fact that orchards can change their susceptibility status in different growing seasons affords the prospect of controlling the problem once the underlying cause of DBD is understood.

Table 1. Average incidence (%) of DBD-type symptoms in fruit from orchards investigated in 2005

<b>Farm reference</b>	<b>No. Orchards</b>	<b>No. Plots</b>	<b>%DBD symptoms</b>
C	4	4	23
H	9	9	3
F	3	12	6
BY	3	3	15
BL	2	4	1
S	1	1	0
G	1	2	5
BI	1	2	0

### *Investigations at trial site reference C*

Least DBD (6%) occurred in fruit from an orchard that received no 'Cultar' or 'Regalis' and most DBD (44%) was recorded in fruit from a different orchard that had

received a 14-day programme of ‘Cultar’ (Figure 1). In a different part of the latter orchard where only two sprays of ‘Cultar’ were applied (followed by ‘Regalis’) the incidence of DBD was reduced to 12%. In another orchard where ‘Regalis’ was applied during flowering the incidence of DBD was high (31%). Interpretation of the effects of spray treatments is confounded by innate orchard differences in susceptibility to DBD. The results implicate ‘Cultar’ use in DBD susceptibility but clearly a high potential can develop where no ‘Cultar’ is applied as indicated by 31% affected fruits where ‘Regalis’ only was applied. Clearly dividing orchards to compare effects of treatments is likely to be much more informative than using different orchards for each treatment.

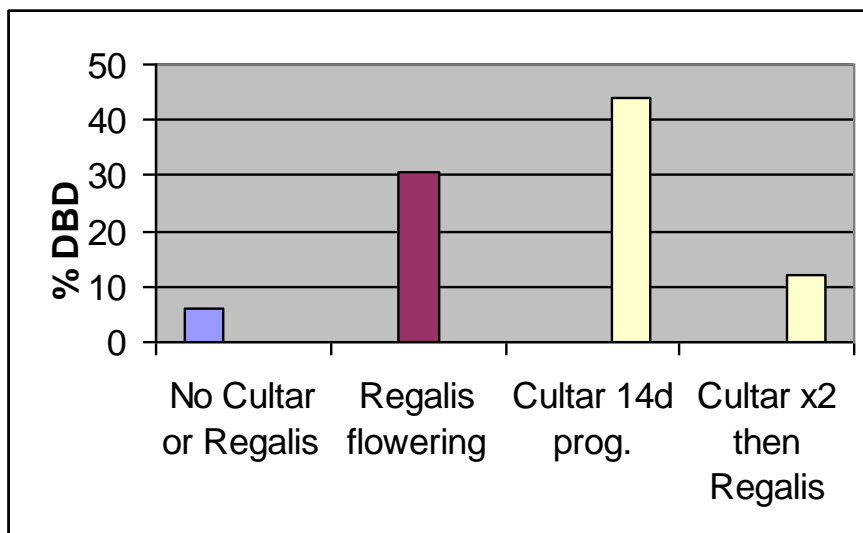


Figure 1. Grower reference C. Alternative growth regulator regimes in different orchards in 2005

*Investigations at trial site reference C*

Using different orchards to compare the effects of different spray treatments is only likely to be helpful if all orchards have the same potential to develop DBD. Experience has shown that this is not the case and that some orchards are consistently high-risk and others low risk. At trial site reference H the number of orchards that received a ‘Stroby’/‘Systhane’ programme (3) was half that which received a

‘Captan’/‘Nimrod’/‘Scala’ programme (6) (Figure 2). Unbalanced comparisons add to the problems of using different orchards for different spray treatments.

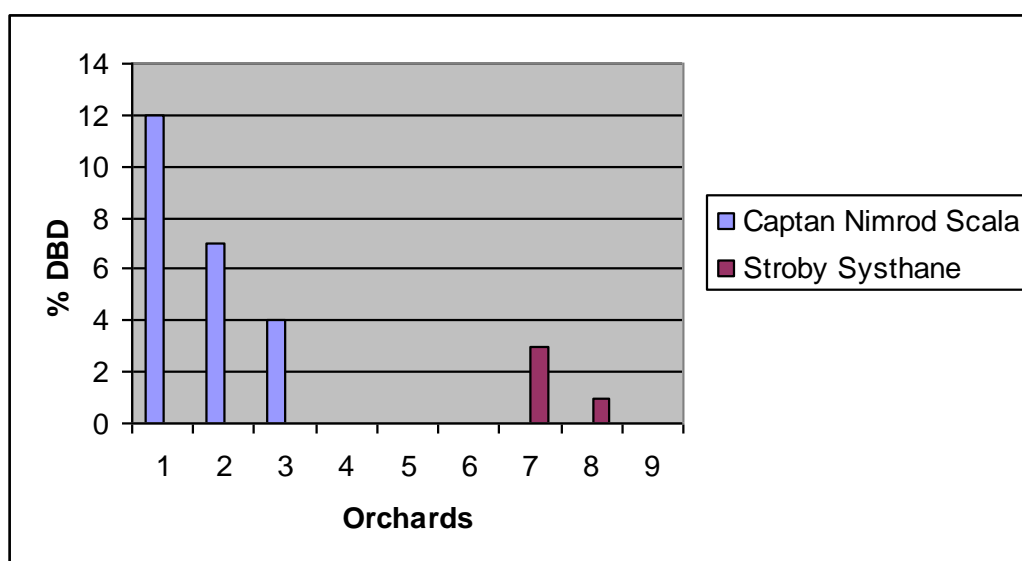


Figure 2. Grower reference H. Early ‘Dithianon’ / sulphur, alternative sprays 15 April -14 May (see legend) then ‘Systhane’ or ‘Topas’

Changing the fungicide programme in the period mid-April to mid-May did not affect the incidence of DBD. The average incidence of DBD in fruit from trees receiving bloom sprays of ‘Systhane’ and ‘Stroby’ (1.3%) was similar to that in fruit from trees sprayed with ‘Captan’, ‘Scala’ and ‘Nimrod’ (3.8%). The average incidence of DBD over the 9 orchards was low (3%) with the highest incidence occurring in fruit from a heavily cropping 4-row bed (12%). It was interesting to note that the 4-row bed orchard was the only orchard to receive two applications of ‘Regalis’ (2.25kg / ha); the remaining orchards were sprayed once (1-1.25kg / ha).

#### *Investigations at trial site reference F*

Sprays additional to the standard programme were applied to complete rows during the bloom period in 3 orchards. Two applications (over-sprayed) of ‘Systhane’, ‘Cultar’/ ‘Regulex’ or ‘Systhane’/‘Cultar’/‘Regulex’ were applied on 3 and 15 May 2005. There was no consistent effect of the over-spray treatments on the incidence of DBD in the stored fruit. In orchard A there was a suggestion that additional ‘Systhane’ increased DBD, but in orchard C all over-spray treatments were associated

with reduced DBD. There was insufficient DBD in fruit from orchard M to determine any treatment effects. The approach taken at this site, i.e. treatment comparisons within orchards was preferable to that adopted at other sites where different orchards received different treatments. However, little is known about the tree-to-tree variation in DBD susceptibility or whether different parts of an orchard are inherently different as regards susceptibility. Consequently, spraying and sampling entire rows of trees may be inadequate for the purpose of comparing treatment effects in commercial orchards. This aspect needs to be taken into account in any future large-scale investigations.

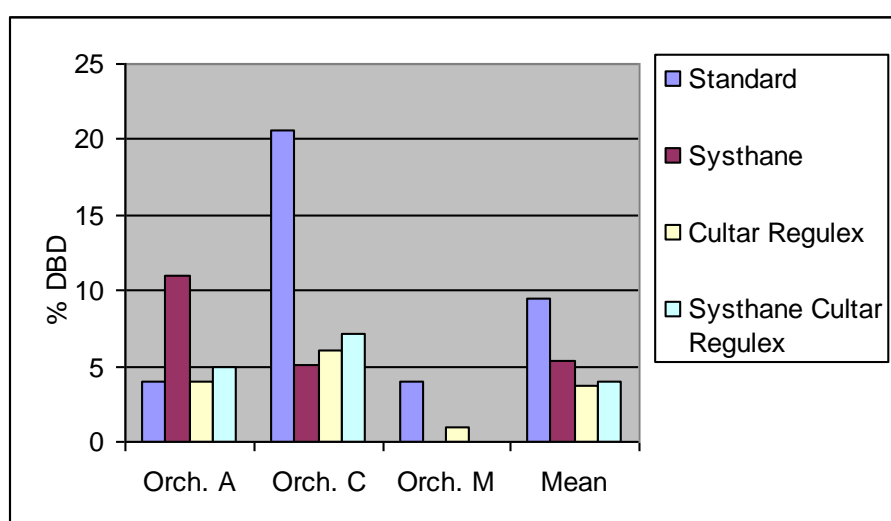


Figure 3. Grower reference F. Over-sprays treatments applied in three orchards (Orch) on 3 and 15 May 2005. Standard is ‘Captan’, ‘Scala’, ‘Nimrod’ and ‘Regalis’

#### *Investigations at trial site reference BL*

Interesting comparisons were made by the grower at trial site BL. A normal programme of sprays (including ‘Cultar’ applied in June) was compared with a programme that omitted ‘Cultar’ and a virtually unsprayed section of orchard (apart from a few late sprays for mildew control). Unfortunately, DBD incidence averaged only 1% and was insufficient to provide any comparisons of the treatments. It was interesting that the grower reported no problem with DBD in fruit harvested in 2005. This compares with major financial losses due to DBD in the previous season.

### *Investigations at trial site reference BY*

There was conflicting evidence from site BY regarding the possible involvement of 'Cultar' application in DBD development. Unfortunately there were no comparisons made within orchards. It is therefore not possible to know whether the recorded level of DBD was associated with differences in the spray programme or due to other orchard factors. Cox apples from three orchards on this site developed DBD in store. However, the apples worst affected (32%) were from a 4-row bed orchard that were not sprayed with 'Cultar' in bloom. The remaining orchards were sprayed with 'Cultar' but developed less DBD (4% and 9%).

### *Investigations at trial site reference S*

The grower at site S reported no previous problems with DBD, despite his farm being adjacent to site BL where serious commercial problems with DBD had occurred, particularly in 2004. Samples from site S in 2005 did not develop any DBD which was consistent with previous commercial experience. The grower at site S has never applied 'Cultar' before the end of May.

### *Investigations at trial sites reference G and BI*

On trial sites G and BI staff from EMR are carrying out trials to minimise pesticide residues in apples in an unrelated project in which normal grower spray programmes are compared with modified programmes where no pesticides are applied after bloom. In fruit from site BI no DBD developed in the stored fruit irrespective of spray programme. In fruit from site G slightly more DBD developed in fruit from the plots receiving the normal programme (7%) than in fruit from the 'zero residue' programme (2%).

### *Mineral composition of fruit*

In the 2002 crop, there was no association between DBD susceptibility and mineral composition of individual apples (see final report on HDC project TF139). A similar exercise was carried out in 2005 with bulked samples of affected and unaffected fruit

from 12 commercial orchards/plots. Again DBD was not related to mineral composition of the fruit at harvest (Fig. 4). It remains possible that DBD could be related to a trace nutrient that has not been considered or to a transient shortage of a particular nutrient(s) during fruit development. These possibilities have not been tested.

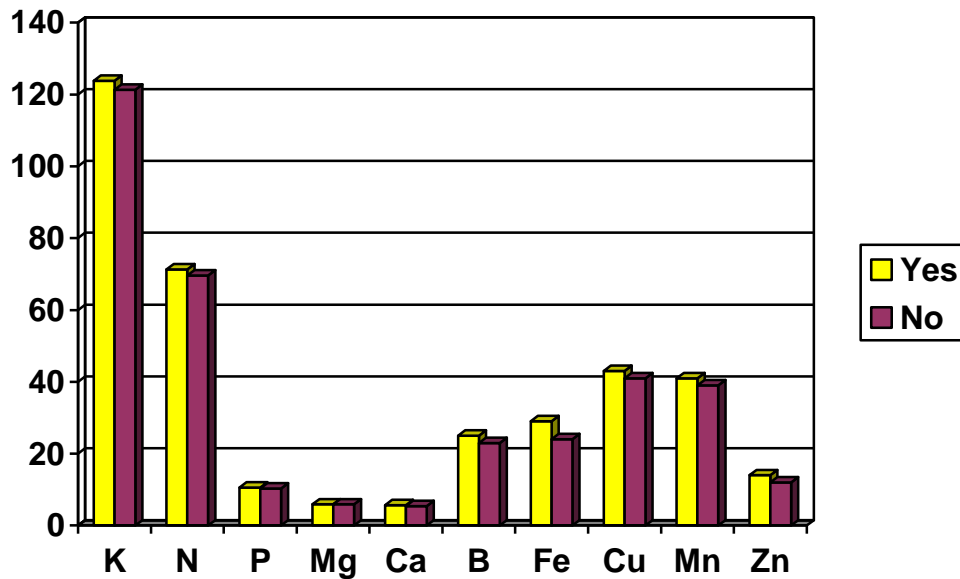


Figure 4. Fruit mineral composition 2005 - averages of samples with (Yes) or without (No) DBD (N, P, K, Ca, Mg - mg/100g; Fe, B - mg\*100; Cu, Mn, Zn - mg\*1000)

## Conclusions

The actual cause of DBD remains obscure and there are no orchard factors that consistently affect susceptibility. The problem is reminiscent of that of bitter pit before an association with calcium nutrition was established with many factors being influential but none consistently so. Once it was clear that bitter pit was only likely in fruit that did not achieve a sufficient threshold level of calcium then the impact of orchard factors could be interpreted.

In 2005-06, DBD was again a serious commercial problem for some growers but it was interesting that some growers with orchards regarded as high-risk for DBD

reported no problems in the 2005 crop. Conversely, there were a number of instances where DBD seriously affected fruit from orchards with no previous history of the disorder.

The association of DBD with particular orchards and the seasonal nature of susceptibility indicate that it will be possible to control the problem once the influence of pre-harvest factors and the underlying mechanisms determining susceptibility are understood.

Targeting the bloom period to compare different fungicide (and growth regulator) sprays in 2005 didn't produce consistent effects. Over sprays of 'Systhane' and/or 'Cultar' applied in early May did not affect DBD incidence compared with a standard programme ('Captan', 'Scala', 'Nimrod' and 'Regalis'). Fruit from orchards that received bloom sprays containing 'Systhane' and 'Stroby' were affected by DBD to a similar extent as those sprayed with 'Captan', 'Scala' and 'Nimrod'. At many sites there was insufficient DBD to make comparisons of different spray treatments.

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

In the longer term a larger scale research project is envisaged to tackle this complex problem, possibly in the form of a HortLINK project sponsored by Defra, HDC and the UK top fruit industry.

### **Technology transfer**

Progress report to growers who attended the MFFS/EMRA Storage Day held at EMR on 30 March 2006.

Discussion of DBD problems in the 2005 crop and the way forward. Meeting held at EMR on 25 April 2006.



## References

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