Project Title	Evaluation of a non-fungicide treatment for the control of *Botrytis cinerea* on stored pears (var.Conference)
Project number:	TF165
Project leader:	Dr Sarah Taylor (HES Ltd)
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Location of project:	HES Ltd., 41 Roebuck Road, Kent, ME1 1UE Innovation Hub, EMR, East Malling, Kent. ME19 6JB
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Key words:	*Botrytis cinerea*, Conference pears, storage, fruit coatings, non-fungicide treatment, Frutavit, potassium bicarbonate.

The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

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Grower Summary

Headline

- Potassium bicarbonate has a limited effect in controlling the extent of rots caused by *Botrytis cinerea.*

- Frutavit does show some promise as a potential non-fungicide replacement to Rovral WP, however, there is the problem of sticky residues on the surface of some fruit.

Background and expected deliverables

Botrytis rot, caused by *Botrytis cinerea*, is the most serious storage disease of pears. Failure to control the disease can result in significant losses in store and a reduced shelf-life. Currently, the disease is effectively controlled by the use of a post-harvest drench of Rovral WP (iprodione). However, there are some indications of likely development of resistance to this chemical and its current off-label approval expires in 2008. There is a real need to develop effective alternative treatments. This project seeks to evaluate the performance of a number of 'non-fungicidal' post-harvest products – Biocoat, Frutavit, potassium bicarbonate and Semperfresh - as potential alternatives to Rovral WP in the control of *Botrytis* rot on conference pears.

The expected deliverable from this project is:

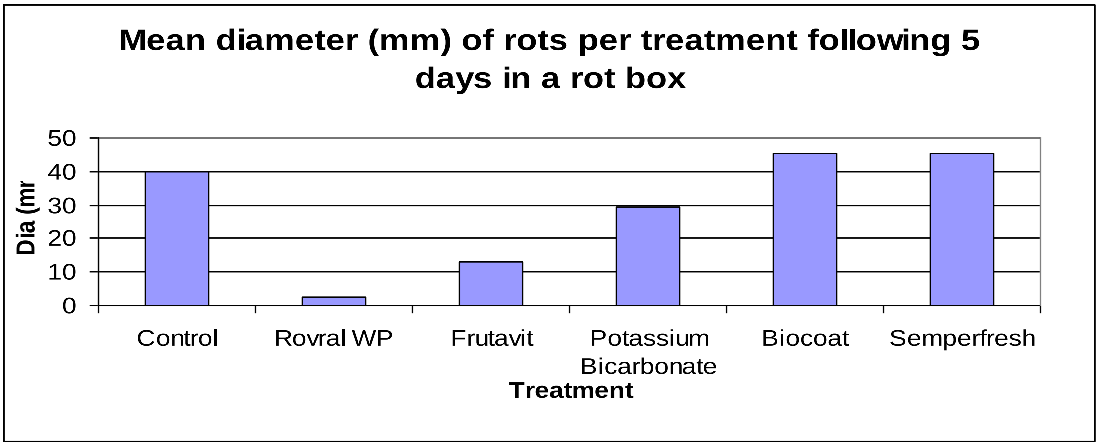
- An evaluation of the efficacy of four non-fungicide products in the control of *Botrytis* rot in stored pears.

Summary of the project and main conclusions

Phase 1: Rot box trial

The first phase of the project tested the performance of the four non-fungicidal post-harvest products (Semperfresh, Biocoat, Frutavit, potassium bicarbonate) to control *Botrytis cinerea* on pears var Conference stored under the extreme conditions (temperature and humidity) of a rot box and in the presence of a high density spore inoculum. Under these conditions, Biocoat and Semperfresh had no effect in reducing the incidence or extent of rots caused by *Botrytis cinerea*. The Frutavit product and to a less extent potassium bicarbonate, reduced the development of *Botrytis* rot on the pears although neither product was as effective as the fungicide product Rovral WP. See Figure (i). However, both these products appeared to give sufficient control in delaying the development of *Botrytis* rot to warrant further investigation in the second phase of the project.

Figure (i). Mean diameter (mm) of rots per treatment following 5 days in a rot box.

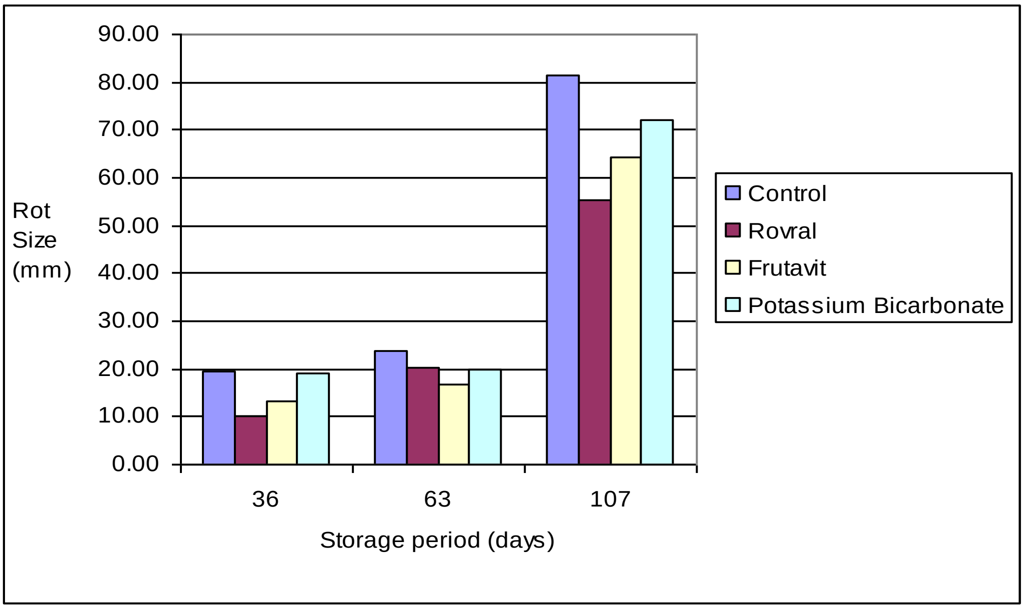


Phase 2. Small-scale trials to compare the efficacy of the post-harvest products in controlling *Botrytis* rot in stored pears.

In the second phase of the project, pears were inoculated with *Botrytis cinerea,* dipped in a tank containing one of the treatments (Frutavit, potassium bicarbonate, Rovral WP or water) for a period of 30 seconds, placed in field crates and stored at 1°C for just over 3 months (107 days). At regular intervals (day 36, 63 and 107) four crates per treatment were removed from store and placed at 20°C for 5 days to simulate shelf-life conditions. The ability of the treatments to prevent infection by *Botrytis cinerea* during cold storage and on the shelf was assessed. The size of the surface lesions (rots) was measured to determine the extent of infection. The presence of mycelium was noted and evidence of spread of disease from one fruit to another was recorded.

Neither Frutavit nor potassium bicarbonate was found to be an effective replacement for Rovral in preventing the infection of pears inoculated with *Botrytis cinerea.* However, there was some indication of partial control by the Frutavit treatment when compared to the control fruit (water), specifically: reduced size of surface rots [see Figure (ii)]; smaller number of mycelium colonies on infected fruit; and reduced spread of disease from one fruit to another. There was, however, an issue with the solubility of the ingredients in the Frutavit preparation which led to a sticky residue on the surface of some of the fruit. Potassium bicarbonate applied with starch (to improve contact with the fruit) showed some limited control in the development of the rots in the latter stages of cold storage, but was less effective than the Frutavit preparation.

Figure (ii). Mean size of rots per treatment for pears held in cold store at 1°C for up to 107 days.



Under less exacting conditions which do not favour the pathogen so highly i.e. low-medium disease pressure and low level of entry points/wounds, Frutavit may provide a reasonable alternative to Rovral WP in terms of slowing down the development of rots and reducing the spread of the disease from one fruit to another in store. However, it is recommended that the third and final phase of this project is delayed until improvements are made to the Frutavit product with regard to the issue of residues observed on some fruit and secondly, the product is either registered for use in the UK on pears or there is some indication that approval is likely.

Financial benefits

If the issues of residues and registration are addressed, the Frutavit preparation could provide an alternative to Rovral in controlling *Botrytis* rot in stored pears under conditions of low-medium disease pressure. The benefits to the industry would be considerable, namely:

- No negative impact on the environment
- Improved safety with regard to application of the treatment and consumption
- Potential for use on organic pears
- Reduced costs
- In addition, there may be added benefits in relation to the quality and shelf-life of the fruit.

Action points for growers

8. None at present.

Science Section

Introduction

Botrytis rot, caused by *Botrytis cinerea*, is the most serious storage disease of pears. The fungus becomes established on the stems and calyxes of pears before harvest and thus has an opportunity to invade the edible fruits while in store. Wounds/mechanical injuries are the main point of entry for this pathogen. Failure to control the disease can result in significant losses in store and a reduced shelf-life. Currently, the disease is effectively controlled by the use of a post-harvest drench of Rovral WP (iprodione). However, there are some indications of likely development of resistance to this chemical and its current off-label approval expires in 2008. With both consumers and the authorities looking for reductions in pesticide usage, there is a real need to develop effective alternative treatments.

In recent years, a number of 'non-chemical' post-harvest products developed to maintain quality and extend shelf-life of fruit and vegetables have entered the market. For some of these products, the manufacturers claim anti-microbial/fungal activity. This project seeks to evaluate the performance of four 'non-fungicidal' post-harvest products as an alternative to the post-harvest drench of the fungicide, Rovral WP (iprodione) in the control of *Botrytis* rot of pears. These products are: Biocoat, Frutavit, Semperfresh and potassium bicarbonate (Table 1). The first three products, which differ in their constituents, have been developed with the aim of maintaining quality and extending shelf-life of fruit and vegetables. Frutavit and Semperfresh have been purported in the literature to have some anti-microbial activity: Frutavit has been shown to be effective against *Botrytis cinerea* on table grapes and Semperfresh has been shown to have some effect in the control of *Monilinia* and *Penicillium* on pears. The ability of the Biocoat product to control fungal diseases of pears is unknown; its inclusion in the project is on the basis that it is an approved organic edible coating with a different mode of action from the Frutavit and Semperfresh products. Potassium bicarbonate was not in the original proposal, but was included in the trials on the basis of recent studies which claim some effect in controlling powdery mildew on a number of horticultural crops.

Table 1. Product details

Product	Company	Description	Availability
Biocoat BC-Z 797	Biocoat Ltd., 23 st .P.O. Box 12, Katzerin, S.C. 12800 Israel	Organic edible coating based on bees wax	Available to growers
Frutavit	Frutavit Ltd., Teradion Industrial Park Misgav Israel	Food grade components	Seeking approval
Semperfresh	Agricoat Ltd., 7B Northfield Farm Great Shefford Berkshire UK	A sucrose ester-based fruit coating	Available to growers
Potassium bicarbonate	Various	Naturally occurring chemical.	Commodity substance approval

The first phase of the project took the approach of using a rot box in order to facilitate a rapid feedback on the performance of the above mentioned products. Artificially wounded and inoculated fruit treated with one of the above products (including a water control and Rovral WP) were held under high temperature and humidity that provide ideal conditions for the growth of *Botrytis cinerea*. Frutavit was found to significantly delay and retard the rate of development of *Botrytis* rot in Conference pears. The treatment was not as effective as the fungicide Rovral WP (iprodione) which gave almost complete control of the disease. Potassium bicarbonate had some impact in reducing the development of *Botrytis* rot although there was some variability within and between replicates.

The second phase of the project sought to compare the efficacy of Frutavit and potassium bicarbonate in the control of *Botrytis* rot in pears stored under semi-commercial conditions, and determine whether there are any signs of the treatment (residues, odours etc) impacting on the quality of the treated pears.

This report presents the findings of the second phase of the project together with a detailed summary and conclusions of the project overall.

Materials and Methods

<u>Source of pears</u>

Conference pears (360 kg) were sourced from Robert Mitchell (Foxbury Farm, Sevenoaks, Kent) and placed in cold store (Jim Mount Building, EMR, East Malling) in air at 1ºC[1].

<u>Disease inoculum</u>

Botrytis cinerea was isolated from decaying naturally infected fruit found in a commercial cold store. The mycelium from the infected fruit were cultured on Potato Dextrose Agar (PDA) in covered Petri-dishes and stored at 4ºC in the dark. One week prior to inoculating the trial fruit, the plates were sub-cultured to provide sufficient inoculum for the trial.

Plaques of agar supporting actively growing mycelium were used as inoculum. This involved cutting standardised discs, 5mm in diameter by 2mm deep, with a cork borer from the leading edge of the colonies.

<u>Wounding and inoculum application</u>

The fruit were artificially wounded by piercing the skin and flesh with a cork screw (diameter 5mm) to a depth of 7mm close to the apex. The mycelial disc was positioned within the wound with the mycelial surface orientated towards the cortex of the fruit.

The fruit was held at 20ºC for one hour before applying the post-harvest treatment.

<u>Application of post-harvest treatments</u>

The treatments were made up in a plastic bin according to the manufacturer's instructions (see Table 2). It was noted in Phase 1 that there was some variability within and between replicates in the performance of the potassium bicarbonate treatment and it was suggested that this might in part be related to poor contact with the fruit. In order to address this, potato starch was included in the treatment as a coating aid. (Starch is approved for use on fresh produce.)

Using a net, pears (24-26 per replicate) were dipped in the bin for 30 seconds whilst gently agitating the solution. The netted fruit were lifted out of the bin, gently shaken to remove excess liquid and allowed to drain before placing in plastic field crates. Each treatment was replicated 12 times.

Table 2. Treatments

Treatment	**Product Rate**
Water (tap): Control	
100% Rovral WP	2.0g/litre
Frutavit	40g/1litre
(a) Potassium bicarbonate + (b) potato starch	(a) 5g/litre + (b) 20g/litre

[1] Fruit were stored at 1ºC as opposed to the recommended storage temperature for conference pears of -1ºC as a result of problems with the refrigeration equipment at the Jim Mount Building.

Cold storage

The treatments were returned to cold storage (air at 1^{o}C) and arranged in a randomized block design on a pallet in the cold store.

Assessments

The treatments (4 crates per treatment) were assessed for the number of fruit infected and size of rots at regular intervals (day 36, 63 and 107) during cold storage.

The distance (mm) between the point of inoculation (close to the apex of the fruit) to the disease front (edge of the rot) was used as a measure of the size of the surface lesion.

The fruit were monitored for signs of mycelium and evidence of disease spread from one fruit to another. Any visible signs (residues etc) of the treatment were recorded.

Shelf-life

Following removal from cold store (at day 36, 63 and 107) the fruit were transferred to ambient conditions (air, 20^{o}C) where they were monitored for the development of *Botrytis* rot. The distance (mm) between the inoculation wound and the disease front was measured on day 5.

Statistical analysis

Mean and standard deviation values were calculated using Microsoft Office Excel. The standard deviation is a measure of how widely values are dispersed from the average value (the mean). Student's t-test was performed to determine if there was a significant difference between the mean value for the control (water) and the mean value for each of the treatments on each sampling date.

Results and Discussion

General

The percentage of fruit showing **no** signs of *Botrytis* rot per treatment on removal from cold store (at day 36, 63 and 107) is presented in Figure 1 together with a table summarizing the mean and standard deviation values for each treatment on each sampling date (Table 3). Figure 2 and Table 4 present the data for the mean size of the rots [measured as the distance (mm) between the point of inoculation to the disease front] per treatment on each sampling date. Photographic plates illustrating the condition of the fruit on removal from cold store at Day 107 (the last sample date) are included in the report.

Figure 1. Percentage of fruit showing no signs of *Botrytis* rot on removal from cold store (1^{o}C).

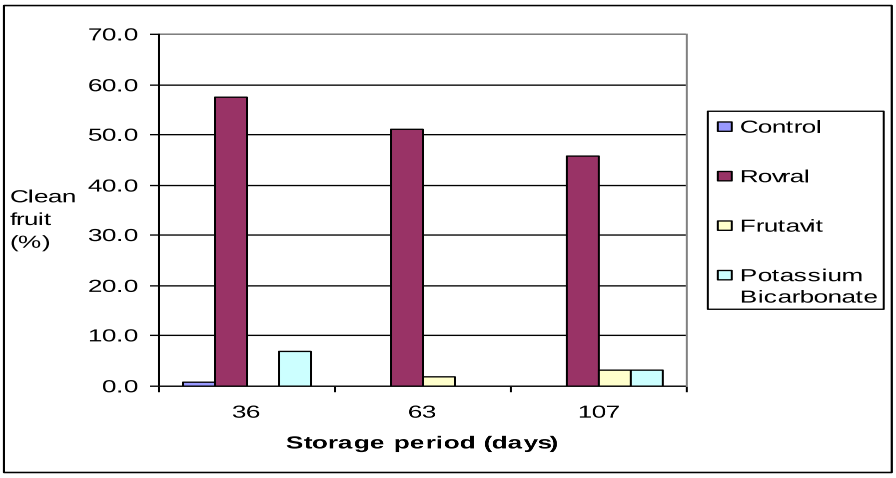


Table 3. Summary data. Mean and standard deviation values for the number of clean fruit per treatment for pears held in cold store up to 107 days.

Treatment	Day 36 Mean	Day 36 Std dev	Day 63 Mean	Day 63 Std dev	Day 107 Mean	Day 107 Std dev
Control	0.8	1.5	0.0	0.5	0.0	0.6
Rovral	57.5	3.2	51.0	38.4	45.8	24.2
Frutavit	0.0	0.0	1.8	0.0	3.1	6.3
Potassium Bicarbonate	6.8	6.2	0.0	0.4	3.1	6.3

On removing from cold store the fruit were held at 20°C for 5 days to establish whether the treatments continued to have any effect in controlling the development of *Botrytis* rot under shelf-life conditions. The data are presented in Figure 3 and Table 5. Please note that for fruit sampled out of cold store on Day 107, that virtually all fruit showing surface lesions collapsed with internal rotting following 5 days on the shelf making it difficult to measure the size of the lesions on the fruit surface.

A full description of the results together with a discussion of the findings are presented in the following paragraphs on a treatment basis.

Figure 2. Mean size (mm) of rots per treatment for pears held in cold store at 1°C for up to 107 days.

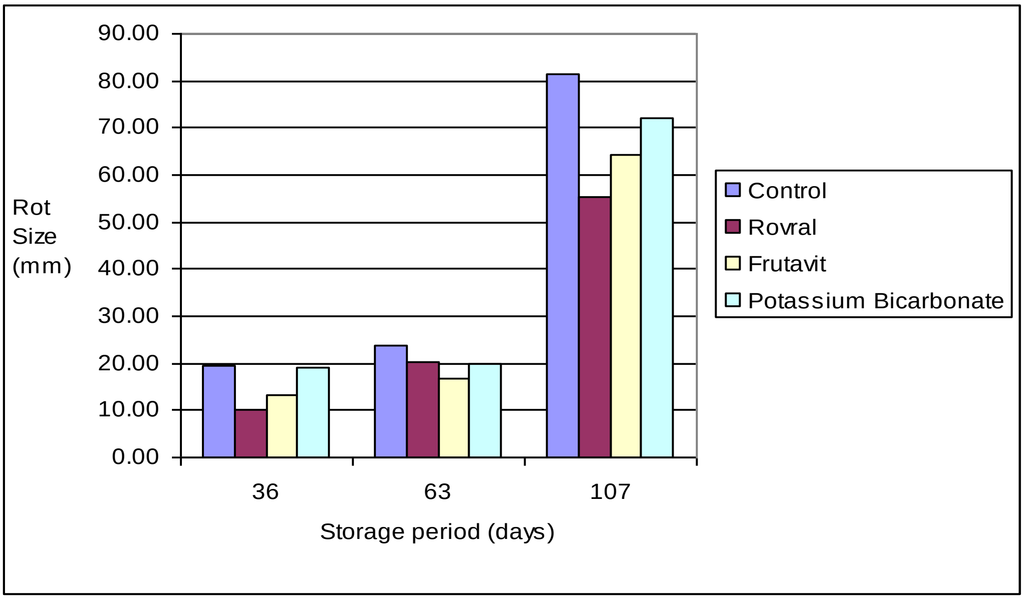


Table 4. Summary data. Mean and Standard deviation values for size (mm) of rots per treatment for pears held in cold store.

Treatment	Day 36 Mean	Day 36 Std dev	Day 63 Mean	Day 63 Std dev	Day 107 Mean	Day 107 Std dev
Control	19.59	0.77	23.85	0.54	81.40	1.92
Rovral	10.07[a]	1.01	20.13[a]	1.04	55.34[a]	1.89
Frutavit	13.36[a]	1.48	16.75[a]	1.42	64.46[a]	2.83
Potassium Bicarbonate	18.98[b]	1.12	19.98[a]	1.69	71.98[a]	3.80

a Mean values which are significantly different from the mean control value ($P<0.001$)

b Mean values which are not significantly different from the mean control value ($P<0.05$)

Figure 3. Mean size (mm) of rots per treatment following 36 and 63 days at 1°C plus 5 days at 20°C.

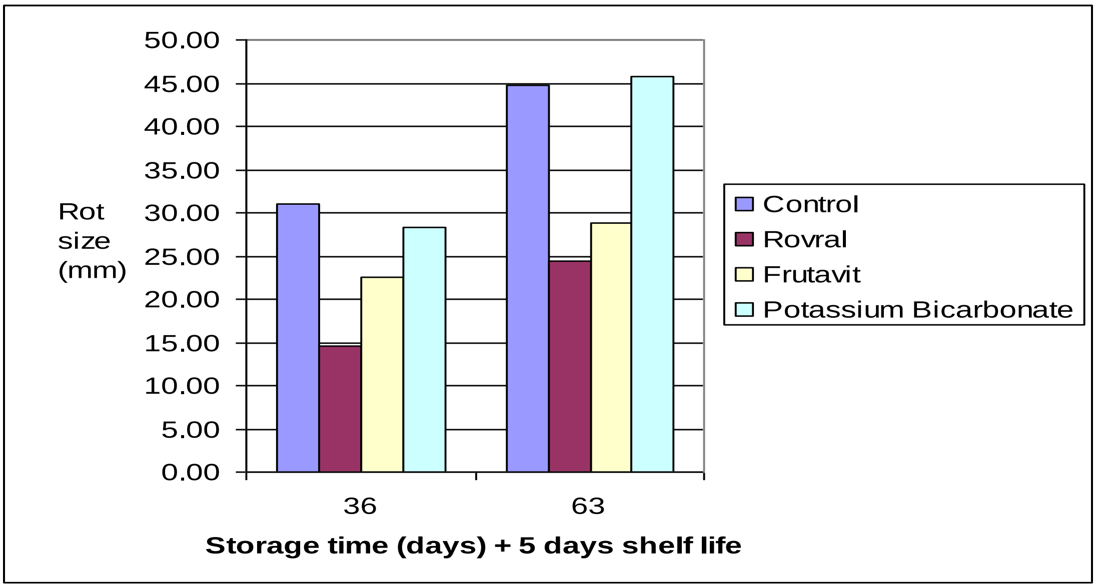


Table 5. Summary data. Mean and standard deviation values for size (mm) of rots per treatment for pears held at 1ºC for 36 and 63 days followed by 5 days at 20ºC

Treatment	Day 36 Mean	Day 36 Std dev	Day 63 Mean	Day 63 Std dev
Control	30.99	1.04	44.72	3.43
Rovral	14.54[a]	1.06	24.43 [a]	3.73
Frutavit	22.55 [a]	0.93	28.74 [a]	5.93
Potassium Bicarbonate	28.23 [b]	0.82	45.82 [b]	1.64

a Mean values which are significantly different from the mean control value ($P<0.001$)

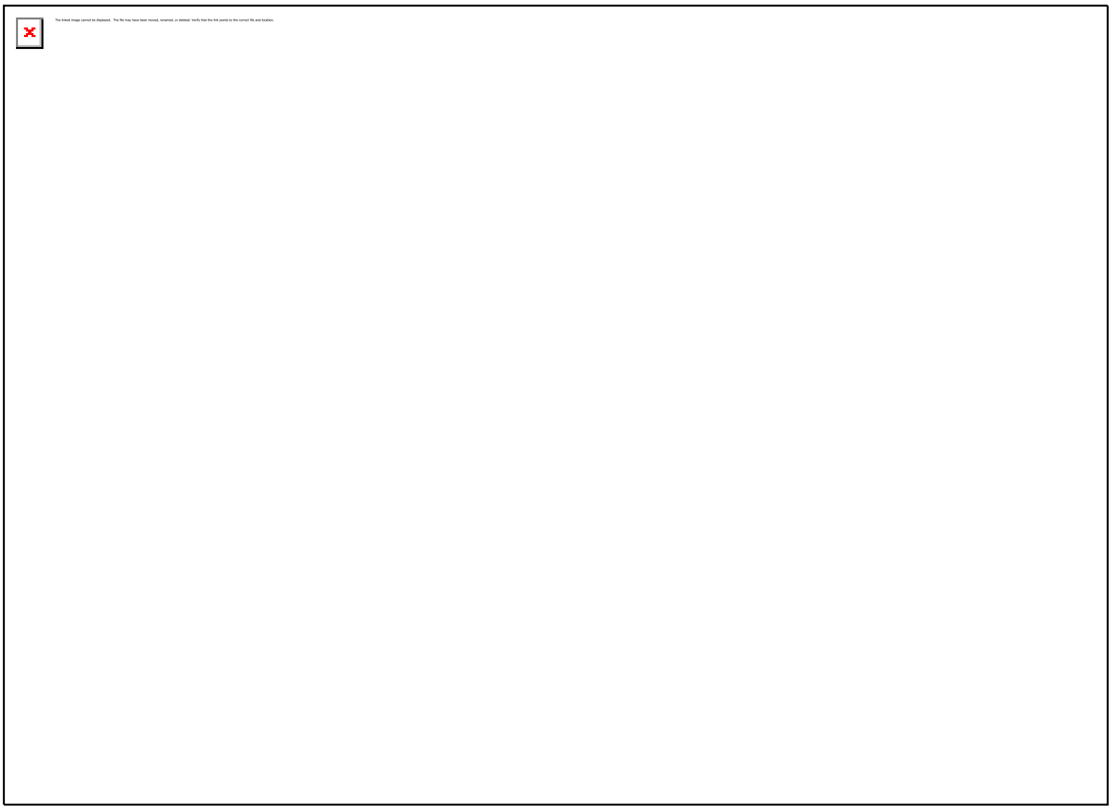
b Mean values which are not significantly different from the mean control value ($P<0.05$)

Water control

Almost all of the water-treated control fruit developed surface lesions by the first sampling date (day 36). For odd fruit that did not develop rots, it was noted that on close inspection of the inoculation sites there were no signs of the mycelium plugs, suggesting these may have dried and fallen out of the wound. Throughout cold storage the surface lesions expanded: between day 63 and day 107 there was a 3.5-fold increase in the mean size of the rots. This might be explained in terms of the reduced resistance of the pears to *Botrytis* with storage age. Small colonies of grey mycelium were seen on the lesions from day 36 onwards - the number of fruit affected increased from 48% to almost 100% by day 107. By this stage, mycelium were seen growing from a number of sites on the surface of the lesion (see Plate 1). Furthermore, 16% of the fruit sampled out of store on day 107 had developed a second lesion as a result of mycelium spreading from one fruit to another. It is known that *Botrytis cinerea* can grow at temperatures below 0ºC and the fact the fruit were held above this temperature helps explain the observed growth of the pathogen and the development of the lesions.

On transferring the fruit (day 36 and 63) to ambient conditions (5 days at 20ºC), the rots greatly increased in size (Figure 3 and Table 5) but the internal condition of the fruit remained firm indicating that the fruits resistance to disease was not compromised. However, fruit taken out of cold store on Day 107 collapsed within 3 days of transferring to shelf-life conditions - the internal condition of the fruit being completely rotten. It would appear that the combination of directly infecting wounds with the pathogen and, storing at 1ºC favoured the development of the disease to such an extent the fruits resistance was completely overwhelmed by the disease.

Plate 1. Control fruit (water) inoculated with *Botrytis cinerea* on removal from cold store (28/02/06). 107 days at 1ºC.

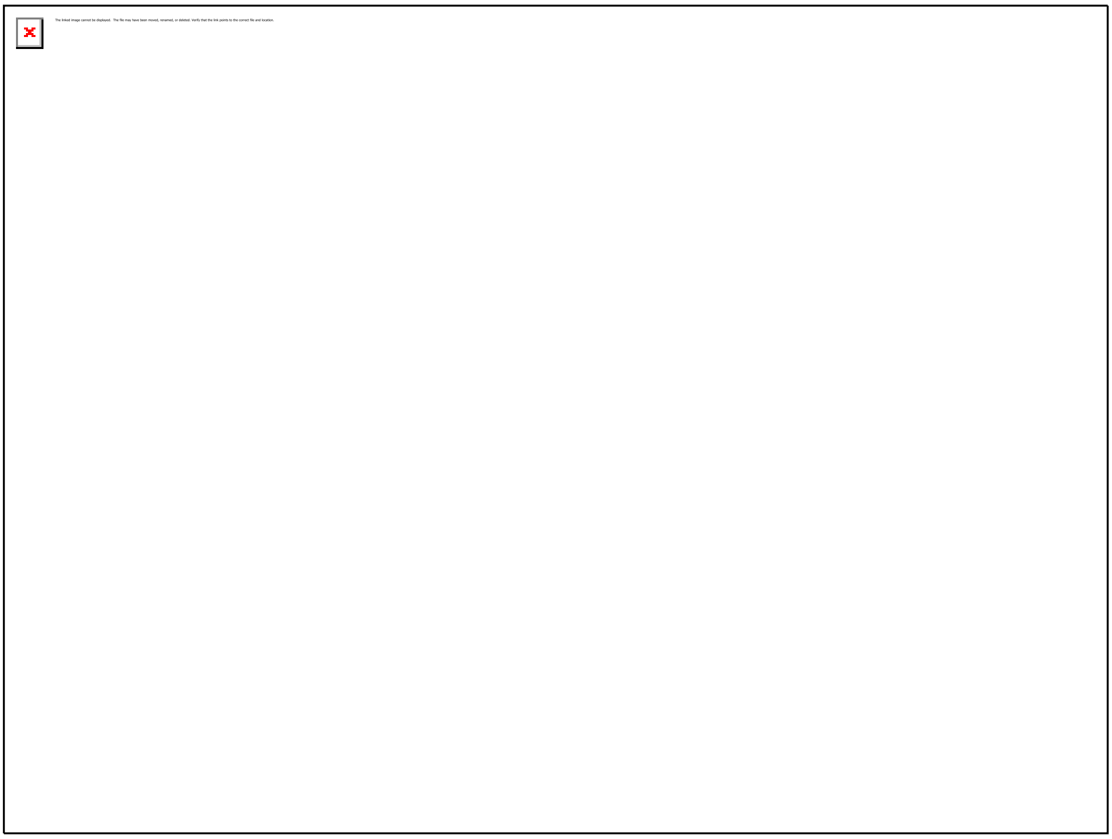


Rovral WP

Rovral WP was found to be the most effective of the treatments in terms of reducing the number of fruit infected by the pathogen and slowing down the development of surface lesions (Figure 1, Plate 2). Following 36 days in cold storage (1ºC) over 57% of the fruit were free of disease, this figure decreased to 45% by day 107. In comparison, only a small percentage of the Frutavit and potassium bicarbonate treatments remained free of disease. On removal from cold storage, there was little evidence of mycelium on the Rovral-treated fruit, except for 3 fruit sampled on day 36, which had a small colony of mycelium at the point of inoculation - possibly due to poor penetration/coverage of the wound by Rovral.

Following 5 days at ambient temperature, the lesions increased in size by 44% and 21% for fruit sampled from cold store at day 36 and 63, respectively. Mycelium was evident on some of the lesions at the end of shelf-life (29% of fruit taken out of store on day 63 subsequently showed signs of mycelial growth on the shelf). Furthermore, nearly 75% of the Rovral-treated fruit taken out of cold store on day 107 developed internal rots on transferring to ambient conditions. This unexpected high level of decay may have arisen as a consequence of the way in which the fruit were infected, namely: the agar plaque supporting the actively growing mycelium may have created a physical barrier which impeded the action of the fungicide, and secondly it may have led to conditions (micro-climate) which favoured the growth of the pathogen.

Plate 2. Rovral WP treated fruit on removal from cold store. 107 days at 1ºC.



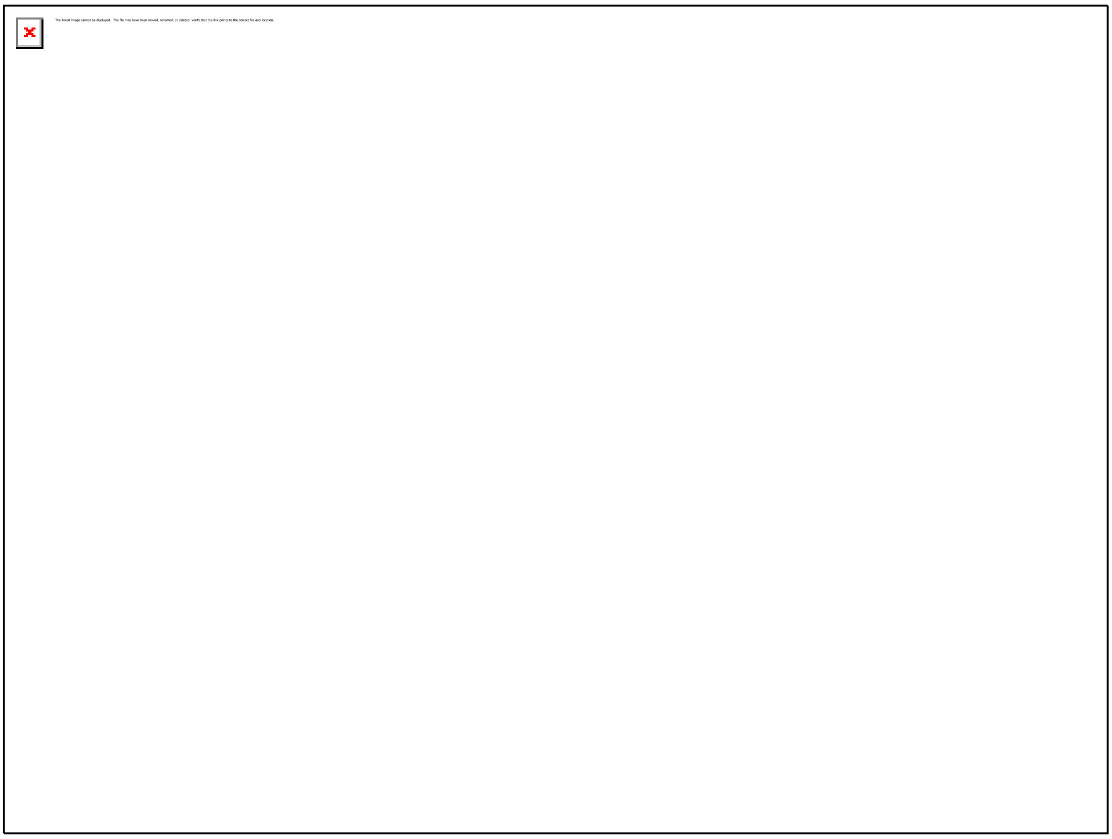
Frutavit

The application of Frutavit to the pears was unable to prevent infection of the fruit from the mycelium plugs, however it did appear to slow down the development of the disease as evidenced by the smaller size of the surface lesions in comparison to the water control (Figure 2 and 3, Plate 3). There was a significant difference between the mean size of the lesions between the Frutavit and control treatments (Table 4), such that following 107 days in cold store the mean size of the rots on the Frutavit treated fruit was 20% less that of the control (water) fruit. The treatment was, however, not as effective as Rovral in this respect. By day 63, almost 63% of the fruit showed signs of mycelium growth at the inoculation site and this increased to 81% by day 107. However, there was no evidence of mycelium nests in any of the crates and no indication of disease spread from one fruit to another.

On transferring the fruitavit-treated fruit from cold store on day 36 and 63 to shelf-life conditions, the rots increased in size by 67% and 72%, respectively over the 5 day period. There, however, continued to be a significant difference between the mean size of the rots for the control (water) and Frutavit treated fruit (Table 5) indicating some control of the disease by Frutavit. For fruit taken out of store on day 107, almost 97% developed internal rots after 5 days at ambient temperature. The Frutavit-treated fruit was clearly overwhelmed by the disease. The reasons for this are probably the same as those put forward for Rovral (see paragraph 33).

There was some evidence of sticky white residues on a small number of fruit. It was noted that whilst making up the solution of Frutavit, a white froth formed on the surface of the water. When the fruit were dipped and removed from the tank some of this froth remained on the fruit and dried leaving the residue. This must be addressed by the manufacturer before this preparation can be used commercially.

Plate 3. Frutavit-treated fruit on removal from cold store. 107 days at 1ºC.

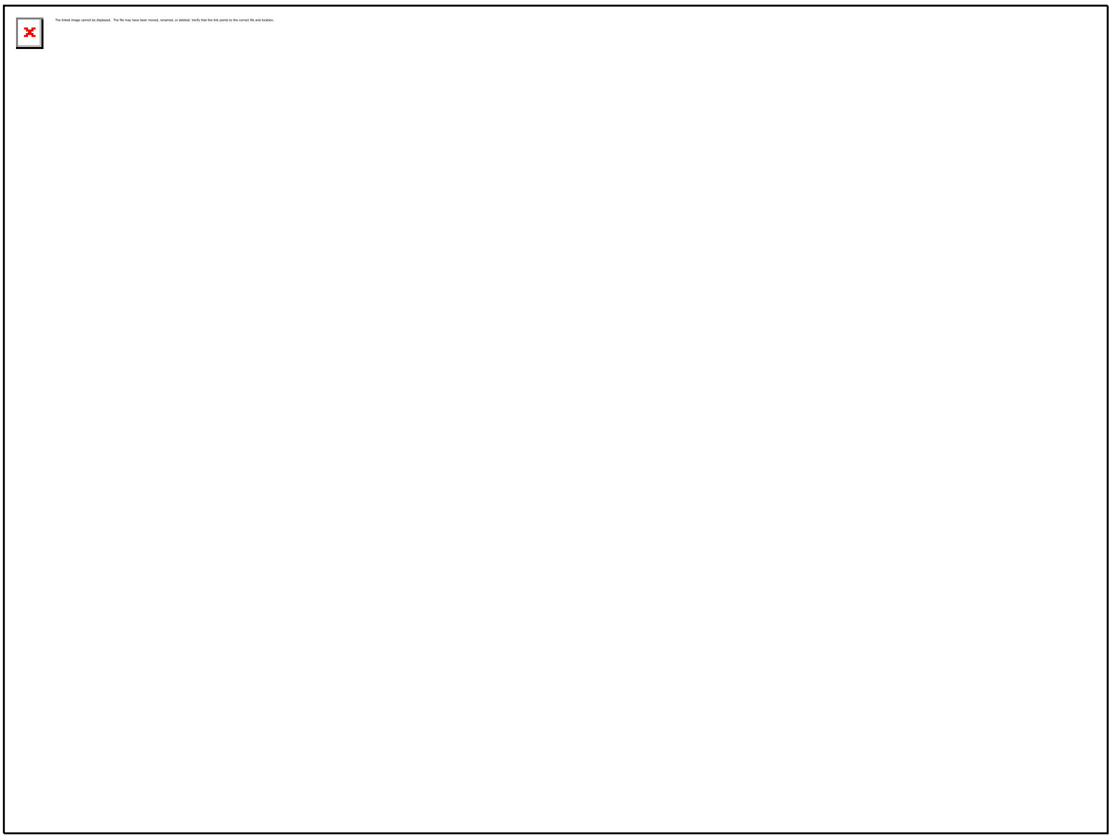


Potassium bicarbonate

Potassium bicarbonate applied in a solution of starch was not effective at preventing infection of pears with *Botrytis cinerea*. All, but a small number of fruit, developed rots in cold store following artificial wounding and inoculation with plugs of mycelium (Plate 4). There was no significant difference between the size of the surface rots for the control and potassium bicarbonate treated fruit following 36 days in cold storage. However as storage time progressed, the potassium bicarbonate treatment did have a statistically significant effect in reducing the rate of lesion development compared to the control fruit (Tables 4 and 5). Mycelial growth was evident at the site of inoculation on 31% of the fruit sampled out of store on day 36 and rapidly increased to 96% of the fruit following 63 days in cold storage. It was also noted that a small number of fruit removed from cold store on Day 107 had internal rots.

On transferring the fruit to ambient conditions the surface rots progressed rapidly, such that there was no significant difference between the size of the rots for the control and potassium bicarbonate-treated fruit. The majority of fruit taken out of cold store on Day 107 collapsed with internal rots on the shelf.

Plate 4. Potassium bicarbonate-treated fruit on removal from cold store. 107 days at 1ºC.



Summary and Conclusions

In the rot box trial, the application of the Frutavit solution to artificially infected pears significantly diminished the rate of development of *Botrytis* rot. The treatment did not, however, prevent infection of the fruit by the pathogen from the inoculated site. It was postulated that the high temperature and relative humidity in the rot box favoured the growth of the pathogen. The performance of Frutavit may be expected to be significantly greater under commercial storage conditions where the growth of *Botrytis* would be reduced Potassium bicarbonate also showed some potential to retard the rate of development of the pathogen, although the results were more variable. It was suggested improvements to how this treatment was applied might increase the effectiveness of potassium bicarbonate in controlling the incidence and/or the rate of spread of the disease.

In the second phase of the project, the two most promising treatments, Frutavit and potassium bicarbonate (applied with starch) were evaluated under semi-commercial conditions against Rovral WP and a water control to establish their effectiveness in controlling *Botrytis cinerea* on stored pears. Neither the Frutavit nor the potassium bicarbonate treatment was able to prevent the infection of wounds in stored pears inoculated with *Botrytis cinerea*. The two treatments were, however, able to reduce the rate of development of *Botrytis* rot during cold storage compared to a water control - the Frutavit treatment having the greater effect, although neither treatment was as effective as Rovral WP. It is worth emphasizing that the Rovral WP treatment itself was only partially successful at controlling *Botrytis* rot as evidenced by the high incidence of internal rots of fruit coming out of store at 3 months. This might be explained by the trial conditions, namely: the wounding and inoculation procedure may have strongly favoured the pathogen; the agar plaque may have formed a physical barrier between the inoculum and the treatments; and lastly the pears were stored at a temperature above that recommended for Conference pears – this would have reduced the storage life of the pears but also permitted the growth of the pathogen. Under these circumstances, the fact there was some indication of control by the Frutavit solution is therefore noteworthy.

Although the application of potassium bicarbonate did have an effect in delaying the development of *Botrytis* rot during cold storage, the level of control is not sufficient to warrant further investigation as a potential pre- or post-harvest treatment for the control of this disease.

In conclusion, the Frutavit coating may offer benefits as a post-harvest treatment in terms of slowing down the development of *Botrytis* rot and providing a barrier to secondary infections arising from the spread of mycelium from one fruit to another. However before this product can be used commercially a number of issues need to be addressed, namely: sticky residues which may have an impact in the way the coating works; and, the product needs to be registered for use by growers. Until these points are resolved, it is recommended that the project is halted.

Technology Transfer

The results from this project are to be published in the July 2006 issue of the HDC News magazine.