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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Previous report	N/A.
Key staff:	Dr Sarah Taylor
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Key words:	*Botrytis cinerea*, Conference pears, fruit coatings, non-fungicide treatment

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

- Of the four non-fungicidal post-harvest applications (Frutavit, BioCoat, Semperfresh and potassium bicarbonate) tested under the extreme conditions of high temperature and humidity in the rot box, Frutavit was the only one able to significantly delay and retard the rate of development of *Botrytis* rot in Conference pears drenched in a high density suspension of *Botrytis cinerea* spores. However, the treatment was not as effective as the fungicide Rovral WP (iprodione) which gave almost complete control of the disease under these conditions.

- Potassium bicarbonate had some impact in reducing the spread of *Botrytis* rot although there was some variability within and between replicates. This may be related in some way to the manner in which the product was applied.

- The post-harvest products BioCoat and Semperfresh had no effect in reducing the incidence or extent of rots caused by *Botrytis cinerea* on fruit held in the rot box.

- The retarding effect of Frutavit and potassium bicarbonate under rot box conditions suggests that under standard cold storage conditions of temperature and humidity, and where the density of *Botrytis* is less, the performance of these post-harvest products should provide a reasonable alternative to Rovral WP.

- It is proposed that this is tested in semi-commercial trials, using both Frutavit and potassium bicarbonate (Phase 2 of the proposal to HDC).

Background and expected deliverables

1. *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. This report presents the findings of Phase 1 of the project which sought to assess the performance of these non-chemical products *versus* Rovral WP and a water-treated control in controlling the development of *Botrytis* rot on artificially infected pears held under the warm and humid conditions of a rot box. The rot box approach was selected in order to facilitate the rapid development of the disease and provide a robust environment in which to evaluate the products over a short period of time.

2. The expected deliverable from this first phase of the project is:

- An evaluation of the efficacy of four non-fungicide products in the control of *Botrytis cinerea* on harvested pears.

Summary of the project and main conclusions

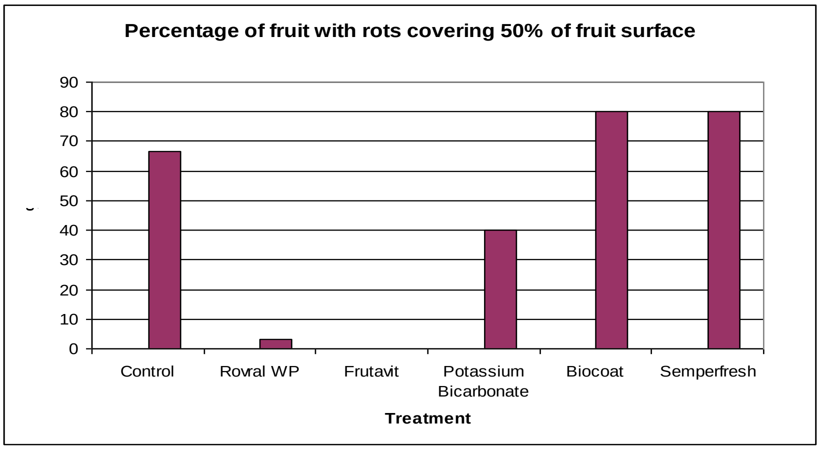
3. On Day 0 of the trial, conference pears were harvested, artificially wounded and then deliberately infected with a high density suspension of *Botrytis cinerea* spores (grey mould). The fruit were treated with one of four non-fungicidal post-harvest products (Semperfresh, Biocoat, Frutavit, potassium bicarbonate) and the fungicide Rovral wp and placed in a rot box (warm and humid conditions). Control fruit were treated with water.

4. *Botrytis cinerea* infections were apparent on all the control (water) fruit within 1 day of exposure to a spore suspension. In comparison, the Rovral treatment showed no signs of *Botrytis* rot (grey mould) on 83% of the fruit following five days in the rot box. *Botrytis* rot developed around the inoculated puncture wounds on all the non-fungicidal (Biocoat, Frutavit, potassium bicarbonate, Semperfresh) treated fruit. For the Biocoat, Semperfresh and potassium bicarbonate treatments this occurred within 1 day – although this was not so advanced for the latter product as for the former ones. For Frutavit the appearance of *Botrytis* rot was not apparent until Day 2.

5. By Day 5, 80% of both the Biocoat and Semperfresh treated fruit were covered in rots which had spread over 50% of the fruit surface, in comparison to 67% of the control fruit and 40% of the potassium bicarbonate treated fruit (see Figure 1). On termination of the trial, Day 5, none of the Frutavit-treated fruit had rots which covered 50% of the fruit surface. The spread/expansion of the rots were less marked on the Frutavit treated fruit which had a final mean rot size of 29mm as opposed to 40, 29, 45 and 45mm respectively for the control, potassium bicarbonate and Biocoat and Semperfresh treatments.

6. To conclude, under the extreme conditions of the rot box, the Frutavit product and to a less extent potassium bicarbonate, reduced the development of *Botrytis* rot on conference pears although neither product was as effective as the fungicide product Rovral WP. However, these products appear to have had sufficient control in delaying the development of *Botrytis* rot to warrant further investigation under less extreme conditions of a cold store. It is therefore proposed that consideration is given to funding the second phase of the project – 'Small-scale trials to compare the performance of the selected post-harvest products (Frutavit and potassium bicarbonate) *vs* Rovral in controlling *Botrytis* on stored pears'.

Figure 1. Percentage of fruit with rots covering 50% of fruit surface on Day 5



Financial benefits

7. The benefits to the industry if one of these products is found to be an effective replacement for Rovral in controlling *Botrytis* rot on pears in store 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

6. 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.

7. 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 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 trial 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 study 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

8. The first phase of the project has taken 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*. This extreme 'hot-house' environment would allow the study to be completed within 5-7 days and would give some indication of the ability of the products to control this disease.

Materials and Methods

9. Conference pears (30kg) were harvested on the 31st August 2005 at Chandler and Dunn (Wingham, Kent). On the same day (Day 0) the fruit were artificially wounded by piercing the skin and flesh with a cork screw (diameter 5mm) to a depth of 7mm at four locations spaced equally around the girth of the fruit (at the widest point). The fruit were then deliberately infected with *Botrytis cinerea* by submerging for 30 seconds in a tank which contained a high density suspension of *Botrytis cinerea* spores. The fruit were removed from the tank using a net and gently shaken to remove excess suspension. The fruit were then transferred to covered plastic containers - the rot boxes (see photo 1) - for two hours at ambient temperature (24 deg. C) before being treated with one of the six treatments (Table 2). The fruit were dipped into the treatments for 0.5 minutes and then allowed to air dry before placing back into the rot boxes. There was a total of 5 rot boxes representing 5 replicates. Each rot box contained 1 kg (6 fruit) of each treatment (a total of 6 kg per box). The treatments were kept separate in the box by placing each treatment on a plate.

10. The fruit were held at 22-28 deg.C and 95-98 RH% for five days. Each day the fruit were photographed and scored for the number of wounds infected. On day 5 the diameter of the rots was measured.

Plate 1. Rot box

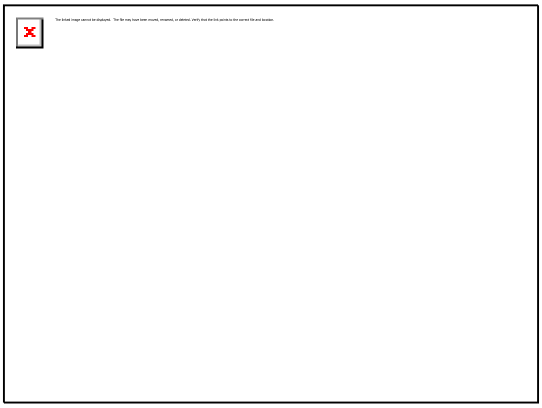


Table 2. Treatments

Treatment	**Product Rate**
Biocoat	Formulation provided
Frutavit	40g/1litre
Semperfresh	200ml/100litre
Potassium bicarbonate	5g/litre
Water (tap)	
100% Rovral WP	2.0g/litre

Results and Discussion

General

11. For each treatment, the percentage of fruit with rots covering 50% of fruit surface on Day 5 is presented in Figure 1. The mean size (diameter, mm) of the rots per treatment on Day 5 is presented in Figure 2. Photographic plates illustrating the impact of the different treatments on the development of *Botrytis* rot are included in the following text.

Figure 1. Percentage of fruit with rots covering 50% of fruit surface on Day 5

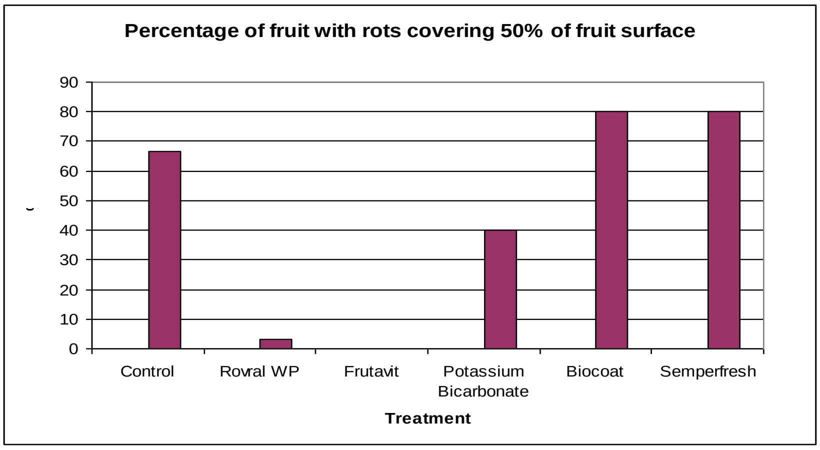
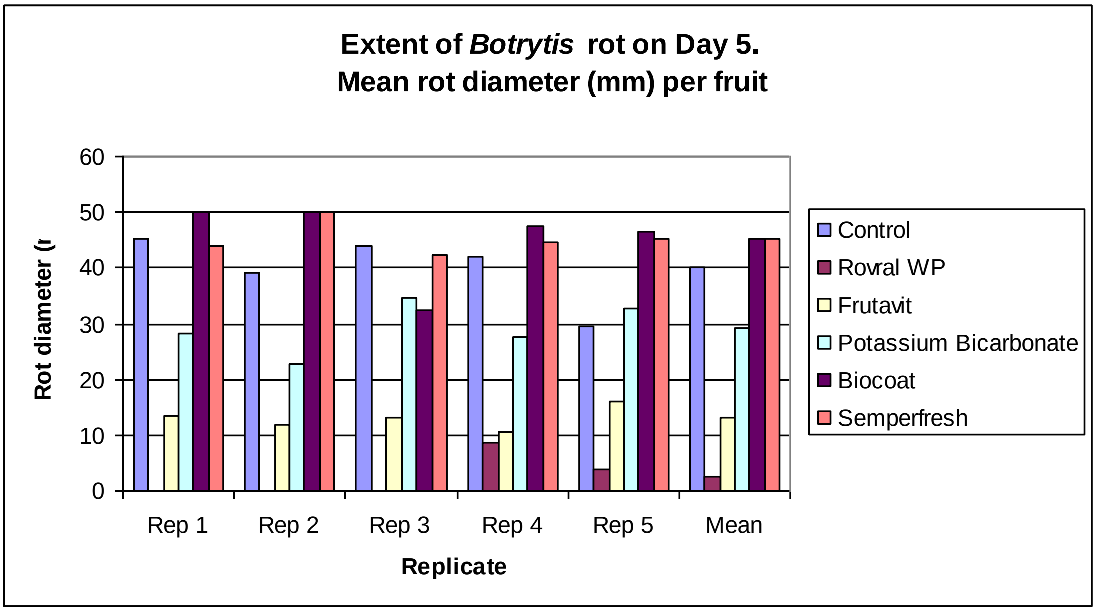


Figure 2. Mean diameter (mm) of rots on Day 5



Rovral WP

12. By the end of the trial on Day 5, 83% of the Rovral-treated fruit remained uninfected. Replicates 1, 2 and 3 were entirely free of *Botrytis* rots. Of the Rovral-treated fruit that developed *Botrytis* rot, 3% (1 fruit from replicate 4) was almost entirely rotten (with over 75% of the surface area showing signs of the rot). The remaining 14% of the fruit had small circular rots around the inoculation sites, typically ranging from 7-14mm diameter in size. Three of the four infected fruit were found in Replicate 5 (see Plate 2). The rots first appeared on Day 2 and slowly increased in size over the course of the trial.

Water control

13. The water-treated control fruit showed signs of rotting at the wound sites from Day 1 of the trial. The rots quickly expanded and by Day 3 adjacent rots had coalesced on many of the fruit. By Day 5, two-thirds of the fruit had rots which covered over 50% of the fruit surface (see Plate 3) and many of these fruit were completely rotten inside. Mycelium growth was seen on the surface of a number of fruit.

Plate 2. Rovral-treated pears. Day 5. Replicate 5

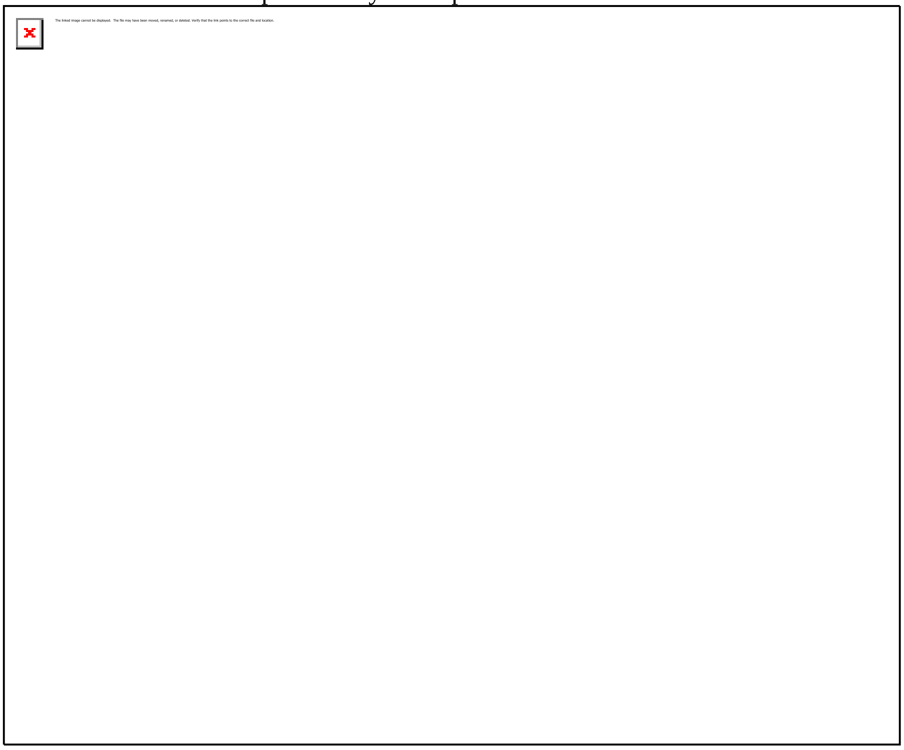
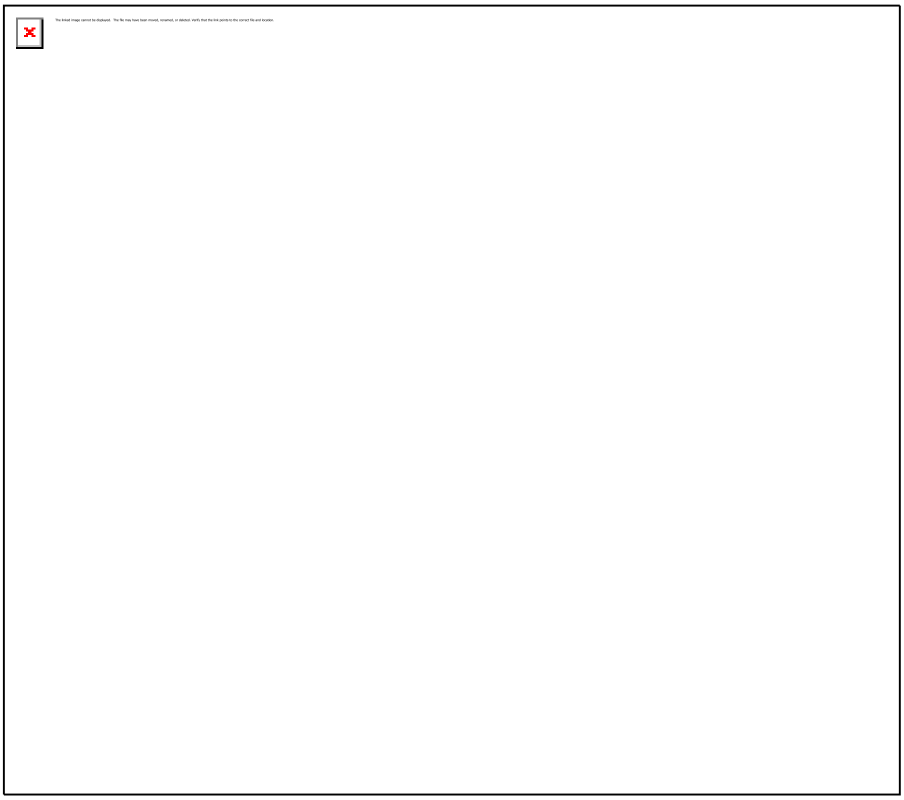


Plate 3. Control fruit (water). Day 5. Replicate 1.



<u>Biocoat</u>

14. The Biocoat-treated fruit inoculated with *Botrytis cinerea* showed signs of rotting at the wound sites from Day 1. These rots quickly expanded, such that by Day 5, 80% of the fruit had rots which covered 50% of the fruit surface and in some cases had almost entirely enveloped the fruit. Plate 4, shows that by Day 4, there was a high level of coverage of rots on the fruit. Indeed the Biocoat-treated fruit seemed to have faired less well than the control fruit with 17% more fruit showing a high level of rot. The mean rot diameter for the Biocoat and Control fruit on Day 5, were 40 and 45mm, respectively. These findings suggest that the organic wax had in some way, directly or indirectly, promoted the development of the rot.

Plate 4. BioCoat-treated fruit. Day 4. Replicate 1

<u>Frutavit</u>

15. Browning around the inoculated wound sites appeared on Day 2 of the trial and by Day 3 all wounds showed signs of *Botrytis* rot. Over the course of the trial the size of the rots gradually increased and by Day 5 the mean rot diameter was 13mm - 32% of the size of that observed on the pear controls (water-treated). None of the Frutavit-treated fruit developed rots which covered 50%+ of the fruit surface. See Plate 5. In comparison to the control pears and the other non-chemical treatments included in the trial, the Frutavit treatment slowed the development of the *Botrytis* rot. None of the rots increased beyond a 34mm diameter. However, all the inoculated wounds became infected with *Botrytis*. Under less extreme conditions (environment and inoculum level) the treatment may prove to be more effective in terms of controlling the development of this disease. It was noted that the fruit remained firm and there were no obvious signs (residues/odours) the fruit had been treated with the formulation.

Plate 5. Frutavit-treated fruit. Day 4. Replicate 5.

Potassium Bicarbonate

16. *Botrytis* rot appeared on the potassium bicarbonate-treated fruit on Day 1 and slowly expanded over Day 2. At this point the size of the rots were typically twice the size observed on the Frutavit-treated fruit but 50 to 100% less than those observed on the control, Biocoat or Semperfresh treated fruit. However, from Day 3 onwards it was noted there was some variation within replicates and between replicates in terms of rot size – and even on the same fruit. By Day 5, the mean diameter size of the rots was 29 mm (double the mean size of the Frutavit rots) but 72% of the size of the control fruit. However, for 60% of the potassium bicarbonate treated fruit the mean diameter size of the rots was 14 mm (comparable to the Frutavit-treated fruit). It may be that the observed variability in the development of rots in the trial is related to the concentration of the salt in the preparation and/or application method.

Semperfresh

17. The results for the Semperfresh treatment were comparable with those obtained using BioCoat. The coating had no impact on the incidence of rots (number of infected sites) and was found to enhance the development of the rots in comparison to the control water-treated fruit. By Day 5 of the trial, 80% of the fruit had rots covering 50% of the fruit surface.

Plate 6. Potassium bicarbonate-treated fruit. Day 4. Replicate 4.

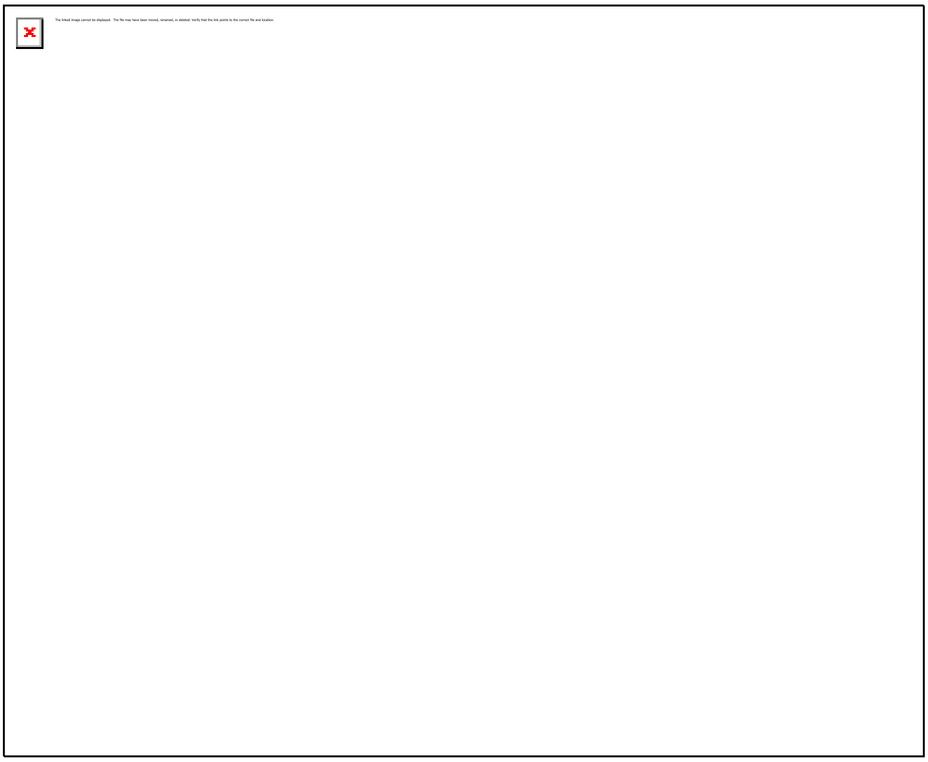
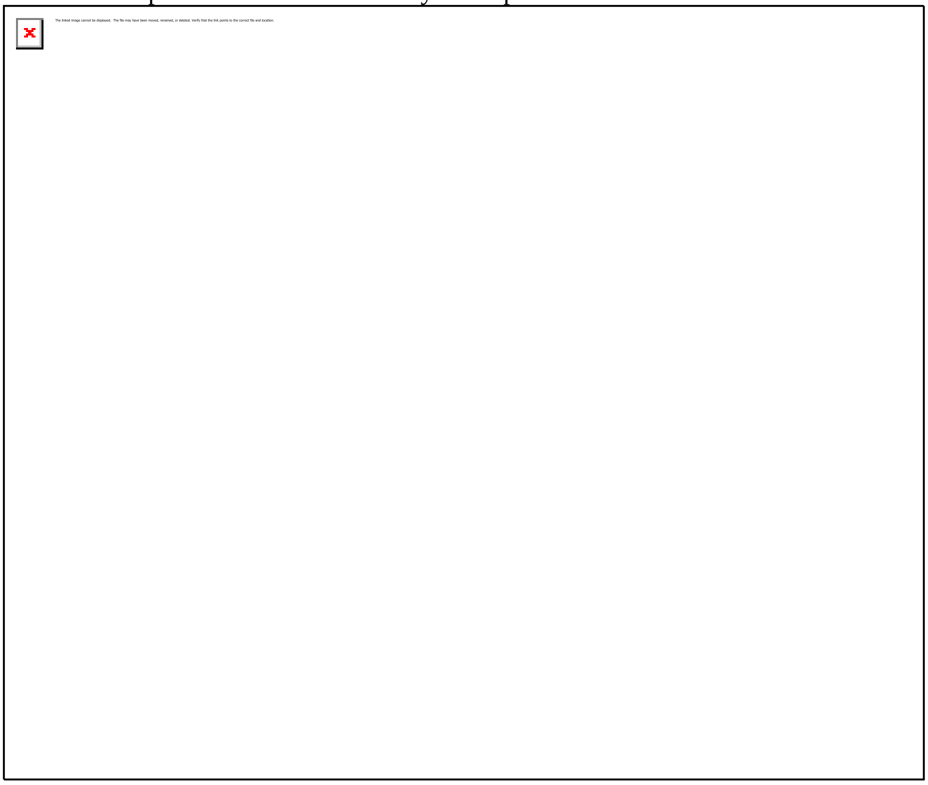


Plate 7. Semperfresh-treated fruit. Day 4. Replicate 5.



Conclusions

18. Whilst the application of the Frutavit solution to artificially infected pears did not have an impact on the incidence – expressed as the number of rots – the preparation significantly diminished the rate of development of the disease. In this trial the density of spore inoculum used was considerably higher than that which would probably occur naturally and, combined with the fact that the conditions under which the fruit were stored were highly favourable to the development of the pathogen, the performance of the treatment would be expected to be significantly greater under standard commercial storage conditions.

19. Potassium bicarbonate also showed some potential to retard the rate of development of the pathogen, although the results were more variable. The indications are that some modification to the way in which this compound is applied may improve its effectiveness.

20. The Biocoat and Semperfresh coatings were found to be ineffective in controlling either the incidence or the rate of spread of the mould. Indeed, under the conditions of this trial, these coatings appeared to facilitate the development of *Botrytis* rot. It should be noted that neither of the manufacturers claim that these products have anti-fungal activity, although there are some reports in the literature that suggest that Semperfresh has some effect in reducing the incidence of other pathogens on stored pears. It may be that the coatings are more effective in terms of providing a barrier to post-harvest infections from mycelium spreading from adjacent fruit in bins/store as opposed to controlling infections which may have been established prior to the application of the coating i.e. originating in the field.

21. In summary, the Frutavit preparation and to a lesser extent the potassium bicarbonate treatment, showed potential for the control of the development of *Botrytis* rot on conference pears. It is proposed that these materials are further tested under semi-commercial conditions as proposed in phase 2 of the project, to include modifications to the method of application of potassium bicarbonate.

Technology Transfer

22. Not applicable at present.