Project Title:	Alternative post harvest treatments for control of *Botrytis* rot in stored pears
Project Number:	TF 135
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Annual Report:	Year 1, November 2003
Previous Reports:	None
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Project Co-ordinator:	
Date Project Commenced:	1 August 2002
Date Project Ends:	31 July 2004
Key Words:	Pear, Botrytis rot, *Botrytis cinerea*, post harvest, stored pears, cold store, biocontrol agent, Rovral WP, iprodione, Serenade, *Bacillius subtilis*, yeast

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The results and conclusions in this report are based on an investigation conducted over one year. The conditions under which the experiment was carried out and the results obtained have been reported with detail and 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.

GROWER SUMMARY

TF 135 Pear: Alternative post harvest treatments for control of *Botrytis* rot in stored pears

Headline

- In the small scale dipping experiment, two alternative products (Serenade and Yeast B) were almost as effective as Rovral WP in reducing *Botrytis* rot

Background and expected deliverables

Botrytis rot is the most important rot in stored pears and failure to control it can result in significant losses in store and limit the storage potential of pears. The fungus gains entry to the fruit at harvest time through wounds and is therefore difficult to control with pre harvest orchard sprays. Currently the rot is well controlled by the use of a post harvest drench of Rovral WP (iprodione) for which an Off-label approval was obtained by the APRC. Recent reorganisations within the chemical industry have made the future of Rovral uncertain. In addition there is increasing public concern about pesticide residues on fruit and post harvest treatments result in the highest residues on fruit, which for Rovral WP on pears is about 2-3mg/kg (MRL = 10mg/kg). Therefore it is important to initiate work to explore alternative treatments. For apples an integrated approach combining cultural control with pre harvest rot risk assessment can be used to minimise losses in store. However because *Botrytis* on pears is mainly a wound pathogen this same approach is not appropriate. Therefore alternative post harvest treatments need to be investigated. In other countries there has been much work on developing biocontrol of storage rot pathogens**.** Examples include Biosave (bacteria), Aspire (yeast) and Yield Plus (yeast). These methods have been most successful with control of wound pathogens such as *Botrytis* and *Penicillium* and therefore may be suitable for treatment of pears post harvest. Some commercially available biocontrol products were evaluated under a previous APRC project (SP 31) mainly on apples. The results on apples were not promising because most apple storage rots arise from orchard infections (eg brown rot, *Nectria* rot, *Gloeosporium* rot) rather than through damage at harvest and biocontrol agents are generally not effective against these types of rot. However they may be effective against pear storage rots. Many other potential biocontrol agents are being developed as post harvest treatments in Europe and may be suitable for pears. In addition to biocontrol agents it may be appropriate to evaluate other fungicides, active against *Botrytis*, and used at lower rates, as post harvest treatment for pears.

The expected deliverables from this work include:

- An evaluation of the efficacy of commercially available biocontrol agents for the control of storage rots, especially *Botrytis* and *Penicillium* rots, in stored pears.
- An understanding of the costs and any differences in practice in using biocontrol agents compared to conventional fungicides

Summary of project and main conclusions

Two experiments were conducted to evaluate the efficacy of biocontrol agents for the control of storage rots of pears, especially *Botrytis* and *Penicillium* rots.

Drenching experiment

In the large scale experiment using bins of pears cv Conference and applying treatments using a commercial drencher, the efficacy of Yield Plus (biocontrol yeast) alone and in combination with 50% Rovral WP was compared with 50% Rovral WP and a water control. The incidence of rotting in the bins assessed the following March was too low (1.6% in the untreated) to draw any meaningful conclusions, but the least number of rots was recorded in the bins treated with Yield Plus + 50% Rovral WP. The trial will be repeated in 2003/2004, using fruit from a different orchard to try obtain a higher incidence of rotting.

Dipping experiment

In the second experiment, pear fruits were artificially wounded and treatments applied by dipping nets of pears into a tank containing the biocontrol treatment to which had been added either *Botrytis* or *Penicillium* inoculum (spores). The efficacy for rot control of the biocontrol yeasts Yield Plus and Yeast B (experimental yeast from Belgium) were compared with Serenade (*Bacillus subtilis*). Rovral WP was included as the standard, together with a water and uninoculated control. When the trial was assessed in January, the percentage of wounds infected by *Botrytis* and the percentage of pears totally rotted was reduced by all treatments. Yeast B and Serenade were as effective as Rovral WP in reducing rotting. None of the treatments were effective in preventing *Penicillium* infection of wounds, but all treatments reduced the percentage of pears totally rotted compared to the water control. However, wounding and inoculation of fruit does favour the pathogen considerably, so any indication of control is worthy of note. The trial will be repeated in 2003/2004.

Financial benefits of the project

- If post harvest biocontrol agents are successful for control of *Botrytis* rot in stored pears, then, provided they could be registered in the UK, they will provide alternative methods to the use of Rovral WP for *Botrytis* control. This will reduce some pesticide use on pears and encourage the use of environmentally friendly methods.
- Take up of the treatment by growers will depend on the availability of Rovral WP and the concerns of the customers with regard to post harvest use of fungicides and residues on the fruit. If these two factors become significant then the availability of an effective biocontrol agent for use on pears would be essential.
- This treatment would be immediately used by producers of organic pears, as there is no alternative treatment for *Botrytis* control.

- Work is needed to further evaluate the products and test new products, but some points can be made regarding the practical use of the yeast products which may present difficulties.

1. The yeast products have to be stored at low temperature in a fridge to maintain viability.
2. The yeasts are supplied as freeze dried products, which means they have to be rehydrated, often using warm water, in a specific way in order to use them. This may present practical difficulties to some growers.

- The cost of the biocontrol products is usually more expensive than the use of Rovral, but if the products prove effective they could be viable alternatives should the use of fungicides as post harvest treatments become unacceptable or the incidence of *Botrytis* isolates resistant to fungicides increase.

Action points for growers

No action points at present as the project is at an early stage.

Science Section

Introduction

Botrytis rot is the most important rot in stored pears and failure to control it can result in significant losses in store and limit the storage potential of pears. The fungus gains entry to the fruit at harvest time through wounds and is therefore difficult to control with pre harvest orchard sprays. Currently the rot is well controlled by the use of a post harvest drench of Rovral WP (iprodione) for which an Off-label approval was obtained for the APRC. Recent reorganisations within the chemical industry have made the future of Rovral uncertain. In addition there is increasing public concern about pesticide residues on fruit and post harvest treatments result in the highest residues on fruit, which for Rovral on pears is about 2-3mg/kg (MRL = 10mg/kg). Therefore it is important to initiate work to explore alternative treatments. For apples an integrated approach combining cultural control with pre harvest rot risk assessment can be used to minimise losses in store. However because *Botrytis* on pears is mainly a wound pathogen this same approach is not appropriate. Therefore alternative post harvest treatments need to be investigated.

In other countries there has been much work on developing biocontrol of storage rot pathogens**.** Examples include Biosave (bacteria), Aspire (yeast) and Yield Plus (yeast). These methods have been most successful with control of wound pathogens such as *Botrytis* and *Penicillium* and therefore may be suitable for treatment of pears post harvest. Some commercially available biocontrol products were evaluated under a previous APRC project (SP 31) mainly on apples. The results on apples were not promising because most apple storage rots arise from orchard infections (eg brown rot, *Nectria* rot, *Gloeosporium* rot) rather than through damage at harvest and biocontrol agents are generally not effective against these types of rot. However they may be effective against pear storage rots. Many other potential biocontrol agents are being developed as post harvest treatments in Europe and may be suitable for pears. In addition to biocontrol agents it may be appropriate to evaluate other fungicides, active against *Botrytis*, and used at lower rates, as post harvest treatment for pears.

Objective

The overall objective is to evaluate commercially available biocontrol agents as post harvest treatments to control storage rots, especially *Botrytis* rot, in stored pears.

Materials and Methods

The biocontrol agents were evaluated for control of *Botrytis* rot and *Penicillium* rot in a large scale experiment, using bulk bins of pears and a commercial fruit drencher and relying on natural disease inoculum and in small scale dipping experiments using wounded netted pears and artifical inoculum.

Drenching experiment

Pears cv Conference were harvested into bins on 28/29 August 2002 from New Gates orchard at HRI-East Malling. The treatments listed in Table 1 were applied to the bins of pears using a Hudson Mark 2 commercial fruit drencher, following HRI Standard Operating Procedure (HRIEF/TE/047). The bins remained under the drencher for one minute and were allowed to drain before being laid out to dry prior to store loading. A water drench was included as a control and Rovral WP (iprodione) as the standard treatment. Treatments were replicated three times. Biocontrol treatments were treated with Jet 5 to kill the yeast prior to disposal of the drench solution down the Sentinel. Bins were stored in air at -1^{o}C until mid March when they were removed and the incidence of rots in the bins recorded.

Table 1: Treatments for bin drenching experiment 29 August 2002

Treatment	Active ingredient	Product rate/1000L
Water	-	-
Yield Plus	*Crytococcus albidus*	1.5kg
Yield Plus + 50% Rovral WP	*C albidus* + iprodione	1.5kg + 1kg
50% Rovral WP	iprodione	1kg

Dipping experiment

Pears

Pears cv Conference were harvested into bins on 28/29 August 2002 from New Gate orchard, HRI-East Malling and placed in store in air at -1^{o}C until needed.

Disease inoculum

Two isolates of *Botrytis cinerea* (R107/01, R 204/01), previously isolated from rotting pears, were cultured on Potato Dextrose Agar (PDA) and placed under UV light to encourage sporulation. Spores were washed off plates into a flask and the volume made up to 800ml. The spore concentration was measured using a haemocytometer slide at 1.1 x 10^5 spores per ml.

Two isolates of *Penicillium expansum* (R188/98, R285/98), previously isolated from rotting pears, were cultured on PDA. Spores were washed off plates into a flask and the volume made up to 800ml. The spore concentration was measured using a haemocytometer slide at 1.21 x 10^7 spores per ml.

Dipping

Two experiments were conducted, one including *Botrytis* inoculum and one including *Penicillium* inoculum.

Pear fruits were damaged by pressing forceps into the fruits at four positions around the pear cheek to a depth of 1cm and placed in nets at 44 pears per net per treatment replicate. The treatments (Table2) were made up, according to instructions on the product label, in a plastic tank. 100ml of inoculum, either *Botrytis* or *Penicillium*, was added to the treatment solution in the tank. The prepared nets of pears were then dipped in the tank for two minutes, gently agitating. They were allowed to drain and

then placed in boxes for storage. The dip solution was sampled before and after the addition of the fungal inoculum. The samples were plated out on PDA to check viability of the biocontrol agent and its effect on the fungal inoculum. Rovral WP (iprodione) at full dose and 50% dose were included as the standards and water dip plus fungal inoculum, and a wounded, uninoculated, included as controls. Each treatment was replicated four times and arranged in a randomised block design in the store. The pears were stored in air at –1°C until the end of January 2003 when rotting was assessed.

Table 2: Treatments for box dipping inoculation (*Penicillium* or *Botrytis*) experiment 31 October – 1 November 2002

Treatment	Active Ingredient	Product rate/L
Uninoculated	-	-
Water*	-	-
Yield Plus*	*Crytococcus albidus*	1.5g
Belgian	*Yeast B*	Exact quantity supplied
Serenade	*Bacillus subtilis*	4.4g
50% Rovral WP	iprodione	1.0g
100% Rovral WP	iprodione	2.0g

*Agral added at rate of 1ml/litre (0.1%)

Results and Discussion

Drenching experiment

The incidence of rotting and *Botrytis* in the bins was very low, even in the untreated (Table 3). Rovral WP (50% dose) mixed with Yield Plus was the most effective overall. However, it is difficult to draw any conclusions from this experiment when the incidence of rotting in the bins was so low.

Table 3 Losses due to rots in Conference pears drenched post harvest with various treatments 2002 and assessed in March 2003

Treatment	Active ingredient	Mean no. rots per bin			
		Botrytis	***Penicillium***	**Brown rot**	**Total rot (% loss)**
Water	-	14.3	1.7	5.0	25.3 (1.6%)
50% Rovral WP	Iprodione	1.0	2.0	4.7	17.3 (1.1%)
Yield Plus	*Cryptococcus albidus*	12.0	0.33	3.0	20.0 (1.25%)
50% Rovral WP +Yield Plus	Iprodione + *Cryptococcus albidus*	3.7	0	2.7	9.0 (0.6%)

Dipping experiment

Viability of biocontrol agent

The yeast products (Yield Plus and Belgian Yeast B) or bacteria (Serenade) were present on the PDA plates from the dip tank samples, indicating that the biocontrol agents appeared to be viable. No *Botrytis* colonies were present on the PDA plates from the dip tank solutions from any of the treatments, compared to the many colonies present on the plates from the water control samples. No *Penicillium* colonies or very few were present on the PDA plates from the dip tank solutions from the Serenade or Belgian yeast B plates, compared to many colonies of *Penicillium* on the plates from the water, Rovral or Yield Plus samples. These observations suggest that the treatments were all effective in suppressing the growth of *Botrytis* on the plates, but only the Serenade or Belgian Yeast B were effective in suppressing the *Penicillium*. Rovral WP is mainly active against *Botrytis* but does have some suppressive effect on *Penicillium*. The biocontrol agents are usually effective against both fungal rots.

Dipping experiment

In January 2003 the pears were removed from store and rotting assessed. In each case the numbers of wounds infected with *Botrytis* or *Penicillium* were recorded and the extent of rotting in the fruit. The percentage of wounds infected with *Botrytis* (Table 4) was significantly reduced by all treatments, except Yield Plus, compared to the water control. The percentage of pears totally rotted (Table 4) was significantly reduced by all treatments, except Yeast B, compared to the water control. Experimental Yeast B and Serenade were as effective as Rovral WP in controlling *Botrytis*. None of the treatments were effective in preventing *Penicillium* infection of wounds, but all treatments reduced the percentage of pears totally rotted compared to the water control (Table 5). Only Yeast B and Rovral at 50 and 100% significantly reduced rotting

The efficacy of the products, especially yeast B and Serenade against *Botrytis* looks promising, as they performed almost as well as the standard treatment Rovral. None of the treatments, even the standard treatment, was effective against *Penicillium*. However, wounding and inoculation of fruit does favour the pathogen considerably, so any indication of control is worthy of note. Serenade (*Bacillus subtilis*) was particularly effective in the tests. However, it is known that *B subtilis* acts as a biocontrol agent by producing antibiotics which act against the fungal pathogens. This product may therefore be unacceptable as a post harvest treatment.

Table 4 % of wounds infected with *Botrytis* and % pears fully rotted following various post harvest treatments.

Treatment	% inoculation points infected with *Botrytis*	% fruit completely rotted
Uninoculated	0	0
Water	66.6	33.5
50% Rovral WP	38.1	11.0
100% Rovral WP	43.1	11.3
Yield plus	56.5	20.9
Yeast B	47.0	24.0
Serenade	38.9	14.0

Table 5 % of wounds infected with *Penicillium* and % pears fully rotted following various post harvest treatments.

Treatment	% inoculation points infected with *Penicillium*	% fruit completely rotted
Uninoculated	0	0
Water	99.9	45.9
50% Rovral WP	98.3	21.3
100% Rovral WP	99.5	14.6
Yield plus	99.5	33.8
Yeast B	97.9	25.0
Serenade	99.6	37.9

Conclusions and Future work

- In the bin drenching experiment,the incidence of rotting was too low to draw any significant conclusions from.
- In the dipping experiment, Serenade and Yeast B were almost as effective as Rovral in reducing *Botrytis* rot.
- None of the treatments were effective in preventing *Penicillium* infection of the wounded pears.

In year two of the project the evaluation of products will be repeated, but including other commercially available biocontrol agents such as Biosave. In addition to the dipping experiment with wounded inoculated fruit, an experiment using pear fruits inoculated with mycelial plugs of *Botrytis* and placed amongst healthy fruit in a box, will be included in order to examine the efficacy of the biocontrol agents in preventing Botrytis spread in store.

Technology Transfer

The results of year 1 of the trial was presented to growers at the Marden Fruit Show / EMRA members day in March 2003.

A summary report of this talk was published in EMRA members day report March 2003.