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Contents

Grower Summary

Headline

- In the small scale dipping experiment, the biocontrol products - Serenade, Yeast B, Biosave 10 and Biosave 11 - were effective in reducing *Botrytis* rot, but were not as effective as the standard fungicide treatment with Rovral WP (iprodione).
- In a large scale drenching trial using bulk bins of fruit, the biocontrol product Yield Plus was almost as effective as Rovral WP in reducing rotting. Biosave 10 was also effective in reducing rotting compared to the untreated control.
- These biocontrol products could provide reasonable alternatives to post harvest fungicides for controlling *Botrytis* rot in stored pears if post harvest fungicides were no longer available.

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 for the APRC. Recent reorganisations within the chemical industry have made the future of Rovral WP 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.

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

In 2002 and 2003 two experiments were conducted to evaluate the efficacy of biocontrol agents for the control of storage rots of pears, especially *Botrytis* and *Penicillium* rots.

Details of biocontrol products evaluated

Product	Active ingredient	Origin	When evaluated
Yield Plus	*Cryptococcus albidus*	Anchor Bio-Technologies, Cape Town, South Africa	2002 and 2003
Yeast B	-	Belgium	2002
Serenade	*Bacillus subtilis*	USA	2002
Biosave 10	*Pseudomonas syringae* strain ESC – 10	Ecoscience, Longwood, Florida, USA	2003
Biosave 11	*Pseudomonas syringae* strain ESC - 11	Ecoscience, Longwood, Florida, USA	2003

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Dipping experiment

In 2002 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 an inoculated water control and an uninoculated water control. When the trial was assessed in January, the percentage of wounds infected by *Botrytis* and the percentage of pears totally rotted were 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. The pears used for this experiment were mature and this combined with the wounding and inoculation of fruit favoured the pathogen considerably, so any indication of control is worthy of note.

In 2003 the trial was repeated. Yield Plus was again used, but Yeast B could not be obtained. Serenade was not included as the bacterium in this product act by producing antibiotics and it was thought unlikely that such a product would ever be registered as a post harvest treatment in the UK. Two commercially available products – Biosave 10 and Biosave 11 (both isolates of the bacteria *Pseudomonas syringae*) from USA were also included. Rovral WP at 100% and 50% of the dose were included as the standards and water and an uninoculated included as controls. When the trial was assessed in March 2004 the percentage of wounds infected by *Botrytis* or *Penicillium* was recorded. The pears used were less mature than those used in 2002, thus rot progression was much reduced and only the wound area infected. The percentage of wounds infected with *Botrytis* was significantly reduced by all treatments, except Yield Plus (80.0% wounds infected), compared to the water control (79.0% wounds infected). Biosave 11 was more effective than Biosave 10 in preventing infection of the wounds by *Botrytis* (12.7% of wounds infected compared to 41.8% of wounds), but neither was as effective as the fungicide Rovral WP at 100% of the dose, where none of the wounded pears were infected with *Botrytis*. Rovral WP at 50% of the dose was almost as effective as the full dose. The percentage of wounds infected with *Penicillium* was significantly reduced by all treatments compared to the water control. Rovral WP at the full dose was most effective (8.7% wounds infected). Both Biosave 10 (25.9% wounds infected) and 11 (27.5% wounds infected) were more effective than Rovral WP at 50% of the dose (44.2% wounds infected). Yield Plus was least effective (53.5% wounds infected). Both Biosave 10 and 11 left obvious brown stains on the fruit which could not be removed by washing or rubbing. No such marking was apparent with Yield Plus.

An additional experiment was conducted to evaluate the efficacy of the biocontrol agents in preventing mycelial spread of *Botrytis* from infected to healthy pears. Pear fruits were inoculated with *Botrytis* and left at ambient temperature to allow the rots to develop. The rotted pear fruits were then placed amongst healthy pear fruits in boxes. Boxes were dipped in the treatments given above allowed to drain and then stored in air at -1^{o}C until March 2004 when the numbers of pears rotted with *Botrytis* were recorded. Each treatment was replicated four times. None of the biocontrol products were effective in preventing spread of *Botrytis* from infected fruit to healthy fruit. The percentage of pears with *Botrytis* was similar or greater in boxes treated with Yield Plus, Biosave 10 or Biosave 11 compared to the water treated control. Rovral WP at 100% or 50% of the dose was almost completely effective in preventing *Botrytis* spread.

The efficacy of the biocontrol products evaluated against both *Botrytis* and *Penicillium* looks promising in preventing wound infection. Biosave 11 appeared to be the most effective, although the presence of the brown stains on the fruit would be a commercial problem. Yield Plus did not perform well in these tests. None of the biocontrol products were as effective as Rovral WP at the full dose. However, wounding and inoculation of fruit does favour the pathogen considerably, so any indication of control is worthy of note. None of the biocontrol agents were effective in preventing *Botrytis* spread.

Drenching experiment

2002

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 was repeated in 2003, using fruit from a different orchard to obtain a higher incidence of rotting.

2003

Pears cv Conference were obtained for the drenching experiment from a mature orchard with a high risk of rotting. The treatments were applied to the bins of pears using a commercial fruit drencher and included Yield Plus, Biosave 10 and water as a control and Rovral WP (iprodione) at 50 and 100% dose as the standard treatment. Bins were stored in air at -1^{o}C until mid April when they were removed and the incidence of rots in the bins recorded. Losses due to rots of more than 19% were recorded in the water treated bins. All the treatments significantly reduced losses due to rots. Most of the losses were due to *Botrytis* rot. Rovral WP at full and half dose were most effective in reducing *Botrytis* rot (3.3-3.5% *Botrytis* rot). Yield Plus (4.5% *Botrytis* rot) was the most effective biocontrol agent. Biosave 10 was also effective in reducing the incidence of *Botrytis* rot (7.0%) compared to the water control (14.1%). The incidence of *Penicillium* was around 2-3% in the water treated control bins and in those treated with biocontrol agents, but was reduced by 50% in bins treated with Rovral WP (50 and !00% dose). The incidence of brown rot was <1% and similar in all treatments.

The biocontrol agent Yield Plus performed better in preventing *Botrytis* infection in the large scale drenching experiment, relying on natural inoculum than in the small scale experiments where *Botrytis* inoculum was introduced. The control achieved was almost as good as that given by Rovral WP at the full dose. Reasons for the poor control in the small scale experiments are not clear. Biosave 10 also gave some control of *Botrytis* rot but was not as effective as Yield Plus. The small scale experiments indicated that Biosave 11 was more active against *Botrytis* and this product may have given better control in the drenching experiments, however there was insufficient Biosave 11 product to use in the larger trial.

Conclusions

- The biocontrol products Serenade, Yeast B, Biosave 10 and Biosave 11 all reduced wound infections by *Botrytis* and *Penicillium* in pears in small scale trials but were not as effective or consistent as the standard fungicide product Rovral WP (iprodione). Yield Plus was ineffective in these small scale tests.
- None of the biocontrol products were effective in preventing *Botrytis* spread from infected to healthy fruit.
- In the large scale bin drenching experiment Yield Plus was almost as effective as Rovral WP in reducing the incidence of *Botrytis* rot; 4.5% and 3.5% Botrytis rot, respectively. Biosave 10 reduced the incidence of *Botrytis* rot compared to the untreated control but was not as effective as Yield Plus.

Financial benefits of the project

- The post harvest biocontrol agents evaluated here gave reasonable control of *Botrytis* rot in stored pears. These treatments, provided they could be registered in the UK, will therefore provide alternative methods to the use of Rovral WP for *Botrytis* control. This will reduce some pesticide use and residue levels 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 in organic production.

- Use of these products will require some changes in practice as detailed below, but this should not 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.
    3. A commercially acceptable method of minimising the obvious brown stains on the fruit from both Biosave 10 and 11 would have to be developed.

- The cost of the biocontrol products is usually more expensive than the use of Rovral WP, 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

There are no biocontrol products for use post harvest on pears registered in the UK so these products cannot at present be used.

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 by the APRC. Recent reorganisations within the chemical industry have made the future of Rovral WP 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.

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 small scale dipping experiments using wounded netted pears and artifical inoculum and in large scale experiment, using bulk bins of pears and a commercial fruit drencher and relying on natural disease inoculum.

Dipping experiment

Year 1 (2002)

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 (Table 1) 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^{o}C until the end of January 2003 when rotting was assessed.

Table 1: 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%)

Year 2 (2003)

Pears

Pears cv Conference were harvested into bins at the end of August 2003 from New Gate orchard, HRI-East Malling and placed in store in air at -1ºC until needed.

Disease inoculum

Four isolates of *Botrytis cinerea* (R28, R18, R192 and R299), 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 1.4×10^6 spores per ml.

Four isolates of *Penicillium expansum* (R130, R318/2/A1, R157/5, R313/1/E2), 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 3.6×10^8 spores per ml.

Dipping – Prevention of wound infection

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 50 pears per net per treatment replicate. The treatments (Table 2) 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 –1ºC until the end of March 2004 when rotting was assessed.

Dipping – Prevention of *Botrytis* spread

One experiment was conducted. Pear fruits were inoculated on opposite cheeks by cutting a flap of skin and placing a mycelial plug of *Botrytis* (either isolate R28 or R192) under the flap and sealing in place with tape. Pear fruits were left at ambient temperature to allow the rots to develop. The inoculated Conference pear fruits (10 per box, 5 of each *Botrytis* isolate)) were then placed amongst healthy pear fruits (approximately 100 fruits) in a box. Boxes were dipped in the treatments in Table 2, allowed to drain and then stored in air at -1ºC until March 2004 when the numbers of pears rotted with *Botrytis* were recorded. Each treatment was replicated four times.

Table 2: Treatments for box dipping inoculation (*Penicillium* or *Botrytis*) experiment 14 and 17 November 2003

Treatment	Active Ingredient	Product rate/L
Uninoculated	-	-
Water*	-	-
Yield Plus*	*Crytococcus albidus*	1.5g
Biosave 10	*Pseudomonas syringae* strain ESC - *10*	1.65g
Biosave 11	*Pseudomonas syringae* strain ESC - *11*	1.65g
50% Rovral WP	iprodione	1.0g
100% Rovral WP	iprodione	2.0g

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

Drenching experiment

Year 1(2002)

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 3 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 3: 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

Year 2 (2003)

Pears cv Conference were harvested into bins on 24/25 September from Red Shed orchard at Foxbury Farm, Stone Street, near Sevenoaks, Kent. The treatments listed in Table 4 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 or bacteria prior

to disposal of the drench solution down the Sentinel. Bins were stored in air at -1°C until mid April when they were removed and the incidence of rots in the bins recorded.

Table 4: Treatments for bin drenching experiment 25 September 2003

Treatment	Active ingredient	Product rate/1000L
Water	-	-
Yield Plus	*Crytococcus albidus*	1.5kg
Biosave 10	*Pseudomonas syringae* Strain ESC 10	1.58kg
100% Rovral WP	iprodione	2kg
50% Rovral WP	iprodione	1kg

Results and Discussion

Dipping experiment
2002
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 WP 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 5) was significantly reduced by all treatments, except Yield Plus, compared to the water control. The percentage of pears totally rotted (Table 5) 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 6). Only Yeast B and Rovral WP 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 WP. None of the treatments, even the standard treatment, were 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 5 % of wounds infected with *Botrytis* and % pears fully rotted following various post harvest treatments. (figures in brackets are angular transformed data)

Treatment	% inoculation points infected with *Botrytis* (angular transformation)	% fruit completely rotted (angular transformation)
Uninoculated	0 (0)	0 (0)
Water	66.6 (55.1)	33.5 (35.2)
50% Rovral WP	38.1 (38.0)	11.0 (19.3)
100% Rovral WP	43.1 (40.9)	11.3 (19.2)
Yield plus	56.5 (48.8)	20.9 (27.2)
Yeast B	47.0 (43.2)	24.0 (29.1)
Serenade	38.9 (38.2)	14.0 (21.1)
SED (15 df)	**(3.6)**	**(3.5)**

Table 6 % of wounds infected with *Penicillium* and % pears fully rotted following various post harvest treatments. (figures in brackets are angular transformed data)

Treatment	% inoculation points infected with *Penicillium*	% fruit completely rotted (angular transformation)
Uninoculated	0	0 (0)
Water	99.9	45.9 (42.6)
50% Rovral WP	98.3	21.3 (27.2)
100% Rovral WP	99.5	22.1 (27.2)
Yield plus	99.5	33.8 (35.5)
Yeast B	97.9	25.0 (29.6)
Serenade	99.6	37.9 (37.9)
SED (15 df)		**(3.9)**

2003

Viability of biocontrol agent

The yeast product (Yield Plus) or bacteria (Biosave 10 and 11) 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 Biosave 10 or Biosave 11 plates, compared to many colonies of *Penicillium* on the plates from the water, Rovral WP or Yield Plus samples. These observations suggest that the treatments were all effective in suppressing the growth of *Botrytis* on the plates, but only Biosave 10 and 11 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 March 2004 the pears were removed from store and rotting assessed. In each case the numbers of wounds infected with *Botrytis* or *Penicillium* were recorded. The pears used in the trial were less mature than in 2002, thus rot progression was much reduced and only the wound area infected. The percentage of wounds infected with *Botrytis* (Table 7) was significantly reduced by all treatments, except Yield Plus, compared to the water control. Biosave 11 was more effective than Biosave 10 in preventing infection of the wounds by *Botrytis*, but neither were as effective as the fungicide Rovral WP at 100% of the dose, where none of the wounded pears were infected with *Botrytis*. Rovral WP at 50% of the dose was almost as effective as the full dose. The percentage of wounds infected with Penicillium (Table 7) was significantly reduced by all treatments compared to the water control. Rovral WP at the full dose was most effective (8.7% wounds infected). Both Biosave 10 and 11 were more effective than Rovral WP at 50% of the dose. Yield Plus was least effective. Both Biosave 10 and 11 left obvious brown stains on the fruit which could not be removed by washing or rubbing. No such marking was apparent with Yield Plus.

None of the biocontrol products were effective in preventing spread of *Botrytis* from infected fruit to healthy fruit (Table 8). The percentage of pears with *Botrytis* was similar or greater in boxes treated with Yield Plus, Biosave 10 or Biosave 11 compared to the water treated control. Rovral WP at 100% or 50% of the dose was almost completely effective in preventing *Botrytis* spread.

The efficacy of the biocontrol products evaluated against both *Botrytis* and *Penicillium* looks promising. Biosave 11 appeared to be the most effective, although the presence of the brown stains on the fruit would be a problem. Yield Plus did not perform well in these tests. None were as effective as Rovral WP at the full dose. However, wounding and inoculation of fruit does favour the pathogen considerably, so any indication of control is worthy of note.

Table 7 % of wounds in Conference pears infected with *Botrytis* or *Penicillium* following various post harvest treatments in 2003, assessed in March 2004. Figures are angular transformed data with % means in brackets

Treatment	**% inoculation points infected with *Botrytis* angular transformed (% means)**	**% inoculation points infected with *Penicillium* angular transformed (% means)**
Uninoculated	0 (0.0)	0.0 (0.0)
Water	62.7 (78.97)	54.3 (65.9)
50% Rovral WP	1.0 (0.03)	41.6 (44.2)
100% Rovral WP	0.0 (0.00)	17.1 (8.7)
Yield plus	63.4 (79.95)	47.0 (53.5)
Biosave 10	40.3 (41.81)	30.6 (25.9)
Biosave 11	20.9 (12.69)	31.6 (27.5)
SED	**6.5 (12 df)**	**7.6 (15 df)**

Table 8 % of pears infected with *Botrytis* following various post harvest treatments in 2003, assessed in March 2004. Figures are angular transformed data with % mean in brackets

Treatment	**% of pears with *Botrytis* Angular transformed data (% mean)**
Uninoculated	1.4 (0.06)
Water	22.3 (14.36)
50% Rovral WP	1.8 (0.09)
100% Rovral WP	2.8 (0.24)
Yield plus	21.8 (13.79)
Biosave 10	30.11 (25.17)
Biosave 11	28.51 (22.78)
SED (18 df)	**2.3**

Drenching experiment

2002

The incidence of rotting and *Botrytis* in the bins was very low, even in the untreated (Table 9). 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 9 Losses due to rots in Conference pears drenched post harvest with various treatments 2002 and assessed in March 2003. (figures in brackets are angular transformed data)

Treatment	**Active ingredient**	**Mean no. rots per bin (angular transformed)**				
		Botrytis	***Penicillium***	**Brown rot**	**Total rot**	**Total rot - % loss**
Water	-	14.3 (21.0)	1.7	5.0	25.3 (30.0)	1.6
50% Rovral WP	Iprodione	1.0 (4.6)	2.0	4.7	17.3 (24.1)	1.1
Yield Plus	*Cryptococcus albidus*	12.0 (15.3)	0.33	3.0	20.0 (24.4)	1.25
50% Rovral WP +Yield Plus	Iprodione + *Cryptococcus albidus*	3.7 (6.5)	0	2.7	9.0 (16.9)	0.6
SED (6 df)		**(9.0)**			**(6.6)**	

2003

In 2003, pears were bought in from a mature orchard, picked late and stored until April 2004 in order to improve the chances of obtaining significant levels of rotting in the trial. The incidence of rotting in the trial is shown in Table 10. Losses due to rots of more than 19% were recorded in the water treated bins. All the treatments significantly reduced losses due to rots. Most of the losses were due to *Botrytis* rot. Rovral WP at full and half dose were most effective in reducing *Botrytis* rot. Yield Plus was the most effective biocontrol agent. The incidence of *Penicillium* was around 2-3% in the water treated control bins, which was reduced by 50% in bins treated with Rovral WP (50 and 100% dose). Much of the *Penicillium* appeared to originate from the bin as most *Penicillium* rots were found where the pears were in contact with the bin. The incidence of cheek rots was <2% in all treatments. These were mainly *Alternaria* sp, *Nectria*, *Gloeosporium* sp and *Stemphylium* sp. The incidence of brown rot (*Monilinia fructigena*) was <1% in all treatments.

In the drenching trial Yield Plus was almost as effective as the Rovral WP drench in controlling rotting, which was in contrast to the dipping experiments where pears were wounded and artificially inoculated. In the latter wound infection by *Botrytis* may be very rapid giving little chance for the yeast in the Yield Plus to act. In the drenching experiment, relying on natural inoculum, wound infection by *Botrytis* may be slower giving greater opportunity for the yeast to act. The Biosave 10 was also effective in reducing rotting and performed similarly in both the dipping and drenching experiments. The active agent in Biosave 10 is a bacteria which may be able to respond more rapidly than the yeast. Brown stains were present on fruit treated with Biosave 10 as was observed in the dipping trial. Biosave 11 was the more active against *Botrytis* in the dipping experiments, but there was insufficient product for this to be included in the drenching experiment.

Table 10 Mean % losses due to rots in Conference pears drenched in various treatments post harvest in 2003 and assessed for rots in April 2004. Figures for *Botrytis* and total rots are angular transformed data with mean % in brackets.

Treatment	Brown rot	*Botrytis*	*Penicillium*	*Mucor*	*Nectria*	*Gloeosporium*	Other cheek	Other eye rot	Stalk rot	Total rot
Water	0.7	22.1 (14.1)	2.3	0.03	0.05	0.02	1.8	0.06	0.08	**25.9 (19.1)**
Yield Plus	0.8	12.3 (4.5)	2.2	0.06	0.04	0.07	1.7	0.02	0.02	**17.8 (9.4)**
Biosave 10	0.6	15.3 (7.0)	3.3	0.1	0.01	0.06	1.6	0.06	0.08	**21.0 (12.9)**
100% Rovral WP	0.8	10.4 (3.3)	1.3	0.1	0.1	0	1.2	0.02	0.08	**15.2 (6.9)**
50% Rovral WP	0.8	10.7 (3.5)	0.9	0.1	0.09	0.06	1.0	0.06	0.03	**14.8 (6.6)**
SED (12 df)		**1.9**								**1.3**

Conclusions and Future work

- In the dipping experiment in year 1, Serenade and Yeast B were almost as effective as Rovral WP in reducing *Botrytis* rot. In year 2, Biosave 10 and 11 were also effective in reducing rotting but much less effective than Rovral WP at the full rate. Yield Plus appeared to be ineffective in this test in both years, despite giving good results in the drenching trial.

- None of the treatments apart from Rovral WP were effective in preventing *Botrytis* spread.

- In year 1 none of the treatments were effective in preventing *Penicillium* infection of the wounded pears. In year 2, where less mature pears were used, both Biosave 10 and 11 reduced wound infection by *Penicillium*, but were not as effective as Rovral WP at the full dose.

- In the bin drenching experiment, the incidence of rotting was too low to draw any significant conclusions from in year1. In year 2, Yield Plus was almost as effective as Rovral WP in reducing rotting. Biosave 10 also reduced rotting but was not as effective as Yield Plus.

- All the biocontrol agents used required special storage (usually at low temperature) and mixing prior to use compared to the fungicides, but were not impractical to use.

- All the biocontrol agents evaluated were not as effective or consistent in their efficacy or convenient as the standard Rovral WP fungicide. However, if pears are to be stored long term without significant losses due to *Botrytis*, biocontrol agents may provide an alternative if use of post harvest fungicides becomes impossible.

- None of the biocontrol agents used are registered for use in the UK

The biocontrol agents tested are all available as commercial products (but not in the UK) apart from the Belgian product (Yeast B), which may soon be available commercially. The active agent in Serenade (*Bacillus subtilis*) acts as an antibiotic and is therefore unlikely to get clearance for use post harvest. Much research is done on biocontrol world wide and therefore more products may become available commercially in the future. Further evaluation of new products may be appropriate, but it is likely that the results will be similar to those obtained here. Unlike fungicides whose performance is more predictable if they are used correctly, the efficacy of biocontrol agents is very reliant on conditions since biological processes are involved. Consequently performance is not always consistent.

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 and 2004. A summary report of this talk was published in EMRA members day report March 2003 and 2004. Results were also presented at an HDC pear meeting in August 2004.

