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PEAR: Evaluation of alternative fungicides to Ronilan applied as pre-harvest sprays or as post-harvest dips for control of Botrytis rot in stored pears.

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AUTHENTICATION

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

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Pear: Evaluation of alternative fungicides to Ronilan applied as pre-harvest sprays or post-harvest dips for control of Botrytis rot in stored pears.

SIX MONTHLY REPORT TO APRC - SEPTEMBER 1994

SP95

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Off-label Approval for the use of iprodione (Rovral) as a post-harvest drench for control of *Botrytis* rot on stored pears

Previous work under Project SP49b established the efficacy of iprodione (Rovral Flo) for control of *Botrytis* rot on stored Conference pears when applied as a post-harvest drench. Data on residue levels of iprodione in pears following treatment were obtained and submitted to the Pesticide Safety Directorate (PSD) to obtain an off-label approval for use of Rovral Flo. This was granted in September 1993. This approval is provisional and expires in September 1998. Correspondence with PSD has established that three further post-harvest residue trials are necessary to obtain permanent off-label approval. This data must be submitted before September 30 1996. In accordance with the PSD data requirements the field study as well as the residue analysis must be done to GLP standard. To achieve this, standard operating procedures must be established. Therefore year one of the study will be dedicated to establishing the necessary standard operating procedures in conjunction with staff at HRI Stockbridge House. The actual residue study will be conducted at harvest 1995. The residue data and the report will then be prepared in time to meet the September 1996 deadline.

The original off-label approval was obtained for Rovral Flo, which is an SC formulation, as this was perceived to be more user friendly than the alternative WP formulation. Unfortunately some growers in 1993 harvest had problems with the flowable formulation during drenching with the drenching solution becoming "lumpy". Such problems were not detected during the original study and have not been experienced by all growers. The cause is not understood, but may be due to the accumulation of leaf debris in the drenching solution reacting with the oily formulation of the Rovral Flo. Therefore in 1994 a case was submitted to PSD for extension of the existing residue data to cover Rovral WP with a view to obtaining an off-label for this formulation. This off-label was granted in September 1994. Further problems have been apparent at 1994 harvest with the Rovral Flo formulation. Therefore only use of the WP formulation will be recommended for harvest 1995 and the residue study planned for harvest 1995 will be conducted with Rovral WP only.

Pear: Evaluation Of Alternative Fungicides To Ronilan Applied As Pre-Harvest Sprays Or As Post-Harvest Dips For Control Of Botrytis Rot In Stored Pears.

SUMMARY:

In a two year study fungicides applied as pre-harvest or post-harvest treatments were evaluated as alternative treatments to Ronilan (vinclozolin) for control of Botrytis rot (Botrytis cinerea) in stored Conference pears. Both Elvaron (dichlofluanid) and Fungaflor (imazalil) reduced rotting in inoculated pear fruits compared to the untreated, but only Rovral (iprodione) gave effective control similar to Ronilan. Captan (captan) and Thianosan (thiram) were ineffective whether applied as pre-harvest or post-harvest treatments. Mildothane (thiophanate methyl) was ineffective in controlling rotting as a benzimidazole-resistant isolate of Botrytis cinerea was used as fungal inoculum.

Residues of iprodione resulting from pre-harvest sprays were lower than those from the post-harvest dips, but both were well below the Maximum Residue Level (MRL) of 10 mg/kg (UK and Codex). Reducing the rate of Rovral or Ronilan reduced the residue in the fruits, and still gave good control of Botrytis rot although at reduced efficacy compared to the full rate.

INTRODUCTION

Botrytis rot of stored pears caused by the fungus Botrytis cinerea is consistently the most important cause of rotting in stored pears. (Berrie, 1989). Before the introduction of post-harvest treatments losses due to Botrytis were considerable and restricted the storage life of pears to December/January. The introduction of benzimidazole fungicides as post-harvest treatments (e.g. Benlate, Bavistin) enabled the potential storage life to be extended to April/May thus increasing the financial returns to growers. Unfortunately Botrytis cinerea readily developed resistance to the benzimidazole fungicides and within 2-3 years the efficacy of the post-harvest drench was much reduced. Studies showed that up to 75 per cent of Botrytis cinerea isolates from stored pears were resistant to benzimidazole fungicides (Berrie, 1989). However, this treatment still gave good control of brown rot (Monilinia fructigena) which can occasionally be responsible for serious losses in store.

Trials with vinclozolin (Ronilan) in the early 1980's demonstrated the effectiveness of this product in controlling Botrytis rot (including those isolates resistant to benzimidazole fungicides) brown rot and suppressing penicillium rot. Since its commercial introduction, rotting in stored pears has been maintained below two per cent losses (Berrie, 1989). Almost all pears and especially those destined for long term storage were routinely treated with Ronilan as the post-harvest treatment. Unfortunately even when the product was applied according to the label recommendation and good agricultural practice, this did not guarantee that the residue was below the required Maximum Residue Level set (UK and Codex). Therefore in September 1990 the Company, BASF, withdrew their support for the post-harvest recommendation on apples and pears. Since then other problems relating to toxicological studies have emerged with the result that the approval for Ronilan has been suspended pending the results of further toxicological studies. Therefore there is now no identified effective treatment available for control of Botrytis rot in stored pears.

Botrytis cinerea is a ubiquitous fungus affecting a wide range of crops with no evidence of host specialisation. Unlike brown rot, Botrytis rot is rarely, if ever, seen on fruit in the orchard prior to harvest although it commonly occurs as a saprophyte on rotting debris on the orchard floor. It appears in store, forming nests of rots if uncontrolled. The fungus usually enters the pear through wounds which probably occur during harvest and which may only be minute blemishes in the skin. Occasionally the fungus can spread into the pear from stalk or calyx end infections, but

the usual entry point is on the cheek of the fruit via wounds. One main source of inoculum is debris, such as leaf, twig and soil picked up on bulk bins during harvest or debris remaining in the bin from the previous season. Drenching in ineffective fungicides can actually increase the risk of rotting by spreading inoculum in the drenching solution. While attention to hygiene, particularly cleaning out bulk bins can reduce the risk to some extent, present harvest techniques make it impossible to avoid the introduction of leaves and other tree debris into the bin.

Because of the nature of the disease post-harvest treatments are likely to be most effective in controlling the rot in store. However, the long term future of such treatments is doubtful. Therefore, any investigation on alternative chemical treatments to Ronilan must examine their effectiveness as pre-harvest treatments as well as post harvest drenches. Since Botrytis rot occasionally appears to originate at the fruit calyx, which could result from infections during blossom, fungicides applied at this time may also give control of the problem and should be evaluated.

The main objectives of the study, which was conducted over two years were therefore as follows:

Experimental Objectives

1. To identify an alternative fungicide treatment to Ronilan (vinclozolin) for control of Botrytis rot on stored pears.

2. To obtain the necessary residue data to enable any effective treatment to receive off-label approval where necessary, for use on pears either as pre-harvest or post-harvest treatments.

3. To assess the efficacy of pre-harvest fungicide sprays in controlling Botrytis rot on pears.

4. To assess the efficacy of fungicide sprays applied at petal fall in controlling Botrytis rot on pears (1992 only).

MATERIALS AND METHODS

The study was in two parts in both 1991 and 1992 investigating pre-harvest fungicide sprays or post-harvest fungicide dips. In 1991 seven different fungicide products were applied as pre-harvest sprays and the same products tested as post-harvest dips. In 1992 the study concentrated on the efficacy of Rovral (iprodione) in controlling Botrytis rot as a pre-harvest spray or a post-harvest dip.

(a) Pre-harvest fungicide sprays

(i) Site

The experiment was located in a mature pear orchard at Court Lodge Farm, East Farleigh, Maidstone, Kent. The orchard consists of the variety Conference on Quince A rootstock. The trees were planted at a spacing of 18 x 12ft with grass alleyways and bare soil herbicide tree strips. The orchard was approximately 40 years old and had a history of significant losses in store due to Botrytis rot.

(ii) Design

The experiment was of a randomised block design with four replicate blocks. A plot consisted of four trees with treatments applied to the centre two trees only, the other trees acting as guards.

(iii) Husbandry

All plots received the same treatments for pest and disease control and nutrition as the rest of the orchard during the growing season.

(iv) Treatments (1991 and 1992)

Table 1. Fungicide treatment and rates of application as pre-harvest sprays in 1991.

Treatment	Product	Active Ingredient	Product rate/l
1	Ronilan (wp)	vinclozolin (50% w/w)	1g
2	Rovral Flo (sc)	iprodione (250g/l)	1ml
3	Mildothane liquid (sc)	thiophanate methyl (500g/l)	1ml
4	Elvaron (wp)	dichlofluanid (50% w/w)	1.5g
5	Elvaron (wp)	dichlofluanid (50% w/w)	2g
6	Captan 83 (wp)	captan (83% w/w)	1.65g
7	Unicrop Thianosan DG (wg)	thiram (80% w/w)	2g
8	Fungaflor (ec)	imazalil (200g/l)	1.5ml
9	Untreated	-	-

(v) Experimental Procedure 1991

Treatments applied are shown in Table 1. Treatments were applied to trees using a motorised air-assisted knapsack sprayer high volume to run-off (1000 l/ha). Each treatment was applied in a three spray programme at timings of six weeks, four weeks and two weeks pre-harvest. At harvest two 30lb boxes of 100 pear fruits were picked from each plot. One box of 100 pear fruits from each plot was inoculated with Botrytis rot by placing ten infected pear fruits (see (b(iv)p. 13) among the 100 healthy fruits in each box. The other box was left uninoculated to rely on natural infection of Botrytis rot from the orchard. Both sets of boxes of pears were placed in commercial pear store at 28-32°F (-2 to 0°C) until mid-March 1992. Numbers of pears rotted with Botrytis and other rots in each box were then recorded.

(vi) Experimental Procedure 1992

After the results in year 1 of the trial, the study in 1992 concentrated on Rovral (iprodione). Spray treatments and timings of application are shown in Table 2. Sprays were generally applied in the six week pre-harvest period, but one treatment included sprays applied at petal fall and repeated one week later. All sprays were applied to trees using a motorised air-assisted knapsack sprayer high volume to run-off (1000 l/ha). At harvest two boxes of pear fruits (50-100 pear fruits per box depending on size) were picked from each plot. One box of pear fruit from each plot was inoculated with Botrytis rot, by placing ten

infected pear fruit (see b(iv) p. 13) among the healthy fruit in each box. The other box was left uninoculated to rely on natural infection of Botrytis from the orchard. Both sets of boxes of pears were placed in a commercial pear store at 28-32°F (-2 to 0°C) and stored until mid-March 1993. Numbers of pears rotted with Botrytis and other rots in each box were then recorded.

Table 2. Fungicide treatments, rates of applications and timings of pre-harvest sprays in 1992

Treatment	Product	Active Ingredient (%)	Product rate/litre	No of sprays	Times of application - weeks pre-harvest
1	Untreated	-	-	-	-
2	Rovral Flo (sc)	ipodione (250g/l)	2ml	5	pf[1], pf +1[2], 5,3,1
3	Rovral Flo (sc)	ipodione (250g/l)	2ml	3	5, 3, 1
4	Rovral Flo (sc)	ipodione (250g/l)	2ml	3	6, 4, 1
5	Rovral Flo (sc)	ipodione (250g/l)	2ml	3	4, 2, 1 day
6	Rovral Flo (sc)	ipodione (250g/l)	1ml	3	4, 2, 1 day
7	Thianosan DG (wg)	thiram (80% w/w)	2g	3	5, 3, 1
8	Mildothane liquid (sc) & Captan 83 (wp)	thiophanate methyl & captan (83% w/w)	1ml & 0.825g	}3	5, 3, 1
9	Captan 83 (wp)	captan (83% w/w)	0.825g	3	5, 3, 1

pf[1] = petal fall

pf + 1[2] = petal fall and 1 week

(b) Post-Harvest Fungicide Dip

(i) Site

The experimental dipping work was carried out at Court Lodge Farm, East Farleigh, Maidstone, Kent. The pears were stored in a commercial air store at the same site.

ii) Design

The experiment was designed as a randomised block with four replicates. A plot consisted of 30lb box of fruit and a block consisted of a bulk bin containing 12 x 30lb boxes of fruit of different treatments, loaded at random.

(iii) Treatments (1991and 1992) - see Tables 3 and 4.

Table 3. Fungicide treatments and rates of application when used as post-harvest dips in 1991.

Treatment	Product	Active Ingredient (%)	Product rate per litre
1	Ronilan FL (sc)	vinclozolin (500g/l)	1ml
2	Rovral Flo (sc)	iprodione (250g/l)	4ml
3	Mildothan Liquid (sc)	thiophanate methyl (500g/l)	2ml
4	Elvaron (wp)	dichlofluanid (50% w/w)	1.5g
5	Elvaron (wp)	dichlofluanid (50% w/w)	2g
6	Captan 83 (wp)	captan (83% w/w)	1.2g
7	Unicrop Thianosan DG (wg)	thiram (80% w/w)	2g
8	Fungaflor (ec)	imazalil (200g/l)	1.5ml
9	Untreated	-	-
10	Water	-	-

Table 4. Fungicide treatments and rates of application when used as post-harvest dips in 1992.

Treatment	Product	Active Ingredient (%)	Product rate per litre
1	Ronilan Fl (sc)	vinclozolin (500g/l)	1ml
2	Ronilan Fl (sc)	vinclozolin (500g/l)	0.5ml
3	Ronilan Fl (sc)	vinclozolin (500g/l)	0.25ml
4	Rovral Flo (sc)	iprodione (250g/l)	4ml
5	Rovral Flo (sc)	iprodione (250g/l)	2ml
6	Rovral Flo (sc)	iprodione (250g/l)	1ml
7	Mildothane liquid (sc)	thiophanate methyl (500g/l)	2ml
8	Mildothane liquid (sc) & Captan 83 (wp)	thiophanate methyl (500g/l) & captan (83% w/w)	1ml + 0.6g
9	Untreated	-	-
10	Water	-	-
11	Pear fruit & orchard debris - undipped	-	-
12	Pear fruit & orchard debris & water dip	-	-

(iv) Production of Botrytis inoculated pears

Pear fruits cv Conference were inoculated by inserting mycelium of Botrytis cinerea (benzimidazole - resistant isolate), previously produced on Potato Dextrose Agar plates, below the skin at two points on the fruit and sealing with lasso tape. The fruits were left for several days at room temperature to allow rot development.

(v) Experimental Procedure 1991 and 1992

Pear fruits cv Conference were harvested from trees which had not received any pre-harvest fungicide sprays apart from sprays applied for scab control up to June. In 1991 100 fruit were placed in 30lb boxes; in 1992 60-100 per box depending on fruit size. Ten inoculated pears (produced as b(iv)) were then placed at random along the healthy fruit in each 30lb box. The boxes of fruit so inoculated were then dipped in fungicide treatments given in Table 3 in 1991 and in Table 4 in 1992. The boxes were dipped by fully immersing in a tank for 1 minute,

allowing the excess fungicide to drain, and the fruit to dry before storage. Water dip treatments were included in both 1991 and 1992. In 1992 treatments which included the addition of orchard debris (leaves/twigs) in place of the inoculated pears were included to investigate this as a source of Botrytis or other fungal rot inoculum. These treatment were either dipped in water only or left undipped. All treatments were then placed in a commercial pear store at 28-32°F (-2 to 0°C) until mid-March the following year. Numbers of pears rotted with Botrytis or other rots were then recorded in each box.

(c) Samples for Residue Analysis

(i) 1991

At the end of the storage period the Rovral (iprodione) Elvaron (dichlofluanid) and Fungaflor (imazalil) treated pears were sampled for residue analysis, from both the pre-harvest orchard treatments and the post-harvest dip treatments. Untreated pear fruit samples were also included. Fifteen sound pear fruits were sampled from two replicates of each treatment using a clean pair of gloves for each replicate and treatment. Sampled pears were placed in a ventilated clean plastic bag clearly labelled with the date and full treatment details. In addition, untreated pears were dipped in Rovral (iprodione) used at the same rate (see Table 3) and following the same procedure as previously. The fruits were allowed to dry and then two replicates were sampled as above for residue analysis. All residue samples were stored at 4°C in a cold room until despatch to Restec Laboratories Limited, Pershore, Worcs, for residue analysis. Residue analysis was carried out to GLP standards. Full details of the results and analytical procedures followed are given in the separate accompanying report.

(ii) 1992 - Residue decline study for iprodione applied as a post-harvest dip

Four separate dipping experiments (Experiments 1, 2, 3, 4 in Table 11) were carried out each of one replicate on pear fruits cv Conference using Rovral Flo (iprodione) at 4ml product per litre of water (treatment 4, Table 4). The dipping procedure was carried out as in (b(v) p.13). Immediately after dipping and after the pear fruits had dried samples of 24 pear fruits were taken from each experiment, and placed in clean ventilated plastic bags each labelled with the experiment number, date and treatment. Untreated pear fruits were similarly

sampled for each experiment. Rovral-treated and untreated sampled pears were immediately despatched to Restec Laboratories Limited, Pershore, Worcs, for residue analysis for iprodione. The remaining Rovral-treated and untreated pears in the four experiments held in new wooden boxes, were placed separately in a commercial pear store at 28-32°F (-2 to 0°C) for storage until March 1993. During the storage period a residue decline study for iprodione was carried out by taking samples of 24 iprodione treated pear fruits from each of the four experiments, following the same procedure as given above, at sample times given in Table 5. Untreated pear fruits were also sampled from the four experiments at the same time. All samples for residue analysis for iprodione were despatched to Restec Laboratories Limited immediately after sampling. Residue analysis was carried out to GLP standards. Full details of the analytical procedures followed are given in the accompanying report.

Table 5. Time of sampling of iprodione-treated and untreated pear fruits from cold store for residue analysis for iprodione, following treatment on 9/9/92

Sample	Time after treatment (weeks)
1	0
2	3 days
3	6 weeks
4	14 weeks
5	26 weeks

(iii) 1992 - Other Residue Analysis

At the end of the storage period Rovral (iprodione) and Ronilan (vinclozolin) treated pears were sampled for residue analysis. Fifteen sound pear fruits were sampled from two replicates of the post-harvest dip treatments 1 and 6 (Table 4) and pre-harvest spray treatments 3 and 5 (Table 2) using a clean pair of gloves for each replicate and treatment. Sampled pears were placed in a ventilated clean plastic bag clearly labelled with the date and treatment. All residue samples were immediately despatched to Restec Laboratoris Ltd for residue analysis. These analyses were not carried out to GLP standards but were included to obtain data on residue levels resulting from different rates of

Ronilan or Rovral used in the dipping experiment, and on the level of residues resulting from pre versus post-harvest chemical treatment.

(d) Statistical Analysis

(i) Inoculated pear experiments
(pre & post-harvest treatments 1991 & 1992)

The numbers of Botrytis rotted pears were recorded and totalled to obtain a mean percentage of rotted pears. Analysis of variance was carried out.

Analysis was straight forward for the % Botrytis rot from pre-harvest fungicide sprays, for 1991/92 (Table 6) and 1992/93 (Table 9).

For data for the % Botrytis rot from post-harvest treatments for 1991/92 (Table 7), treatments 1 and 2 were markedly different from other treatments and were thus excluded from the analysis. Calculated SED are only appropriate for treatments 3-10.

Similarly for data for the % Botrytis rot from post-harvest treatments for 1992/93 (Table 10), the treatment values fell into two distinct groups (Treatments 1-6, 11-12 and Treatments 7-10) and analysis of variance was therefore done on these groups of treatments separately. Thus two SED are provided appropriate to the two groups.

(ii) Uninoculated pears experiment
(pre-harvest treatments 1991 & 1992)

The level of rotting in these experiments was too low to obtain meaningful data for statistical analysis.

RESULTS

(a) **Meteorological Data** (Orchard)

The monthly mean maximum and minimum temperature and rainfall recorded for East Malling (nearest Meteorological station approximately 5 miles distant) for the orchard experimental period are given in Appendix 1.

(b) **Cold Store Temperature Records**

The cold store 17 and store 12 respectively mean maximum and minimum temperatures recorded for the storage period during 1991/92 and 1992/93 are given in Appendix 2.

The store was loaded on 18/9/91 with the store temperature at a mean of 52.6°F. After 6 days the mean temperature was reduced to 40°F. The recommended time to achieve the 40°F is 5 days. The fruit temperature of 30°F (-1°C) was finally achieved after 14 days, 4 days later than the recommended time. In 1992 the store was loaded on 10/9/92. The fruit temperature of 30°F (-1°C)was achieved more rapidly than in 1991 and nearer the recommended total time of 10 days. (5 days to 40°F, 10 days to 30°F.)

(c) **Pre-Harvest Fungicide Sprays 1991/92**

The pears were removed from store and assessed for rotting on 12/3/92. The percentage of Botrytis rot recorded for inoculated and uninoculated treatments is shown in Table 6. The level of Botrytis rot in the uninoculated treatment was very low with no rotting recorded in many boxes. The introduction of Botrytis rotted fruit into the boxes of pears resulted in a high level of Botrytis rotted pears resulting from contact spread of the Botrytis. None of the treatments prevented spread of Botrytis rot. The best control was achieved by Rovral which reduced rotting to 18% compared to 50.3% in the untreated. Other rots recorded included Potebniamyces discolor (stalk rot), and Nectria galligena (eye rot), but these were only at trace levels.

(d) **Post-Harvest Fungicide Dip 1991/92**

The percentage of Botrytis rots recorded for inoculated samples is shown in Table 7. Botrytis cinerea was the main rot recorded apart from trace levels of Potebniamyces discolor and Nectria galligena (eye rot). Fungaflor, Elvaron, Ronilan and Rovral significantly reduced rotting compared to the untreated.

Ronilan and Rovral almost completely prevented spread of Botrytis rot, and were significantly better than the other fungicide treatments.

Table 6. Mean percent Botrytis rot in pear cv Conference treated pre-harvest with fungicide sprays and either inoculated or uninculated with Botrytis and stored until 12/3/92.

	Mean % Botrytis Rot	
Fungicide Treatment	**Uninoculated**	**Inoculated**
1. Ronilan	0	30.0
2. Rovral	0.75	18.0*
3. Mildothane	1.75	39.3
4. Elvaron (1.5g/l)	0	51.3
5. Elvaron (2.0g/l)	0	36.0
6. Captan	3.3	56.0
7. Thianosan	0	52.5
8. Fungaflor	0	58.8
9. Untreated	0.3	50.3
SED (24df)	-	12.2

* significantly different from untreated ($P \leq 0.05$)

Table 7. Mean percent Botrytis rot in pear cv Conference inoculated with Botrytis and treated post-harvest with fungicide dips and stored until 12/3/92.

Fungicide Treatment	Mean % Botrytis Rot.
1. Ronilan	0.5*
2. Rovral Flo	2.5*
3. Mildothane	53.3
4. Elvaron (1.5g/l)	33.5*
5. Elvaron (2.0g/l)	28.8*
6. Captan	70.8
7. Thianosan	57.8
8. Fungaflor	19.5*
9. Untreated	65.0
10. Water	59.0
SED (24df) (treatments 3-10 only)	12.3

* Significantly different from untreated ($P \leq 0.05$)

Table 8. Chemical residue mg a.i./kg in pear fruits from pre or post-harvest fungicide treatments 1991/92.

Chemical Residue A.i. Mg/Kg

Fungicide Treatment	Active Ingredient		After 6 months storage in Commercial pear store at -1.1°C (30°F) Pre-Harvest Sprays	Immediately post-dipping (March 1992) Post-Harvest Dip	Post-Harvest Dip	Untreated	Maximum Residue Level mg/kg
2. Rovral	iprodione	R1	1.24	3.84	2.05	<0.2	10.0
		R2	1.21	3.87	1.85	<0.2	
4. Elvaron (1.5g)	dichlofluanid	R1	0.53	2.34	-	<0.1	5.0
		R2	0.46	2.06	-	<0.1	
5. Elvaron (2.0)	dichlofluanid	R1	1.04	3.27	-	<0.1	5.0
		R2	1.48	3.78	-	<0.1	
8. Fungaflor	imazalil	R1	<0.1	3.39	-	<0.1	5.0*
		R2	<0.1	4.37	-	<0.1	

* No MRL for imazalil given for pears, but 5.0 mg/kg specified for citrus fruit.
R = Replicate

(e) **Residue Analysis 1991/92**

The results of the analysis for residues of iprodione, dichlofluanid and imazalil carried out on pear fruits after storage are given in Table 8. In all examples given, the residue resulting from the single post-harvest dip was two to three times greater than from a programme of three pre-harvest sprays. The residue resulting from Fungaflor (imazalil) applied as three pre-harvest sprays was undetectable after 6 months storage. All residue levels detected were below the maximum residue level set (UK and Codex) for pears for the fungicides tested. Residues of the chemicals were below the level of detection in the untreated pear fruits.

(f) **Pre-Harvest Fungicide Sprays 1992/93**

The pears were removed from store and assessed for rotting on 11th March 1993. The percentage of Botrytis rot recorded for inoculated and uninoculated treatments is shown in Table 9. As in the previous year, the level of Botrytis rot in the uninoculated treatment was very low with no rotting recorded in many boxes of fruit. Evaluation of the efficacy of the treatments applied at petal fall in preventing Botrytis infection of fruit was therefore not possible. The introduction of Botrytis- rotted fruit into the boxes of pears resulted in a high level of Botrytis rot. None of the fungicides applied as pre-harvest sprays prevented spread of Botrytis rot. Rovral sprays reduced rotting ($P \leq 0.05$) compared to the untreated with sprays applied the day before harvest resulting in the greatest reduction in rotting. Other rots recorded included Potebniamyces discolor (stalk rot) and Nectria galligena (eye rot), but only at trace levels.

Table 9. Mean percent Botrytis rot in pear cv Conference treated pre-harvest with fungicide sprays and either inoculated or uninoculated with Botrytis and stored until 11/3/93.

Fungicide Treatment	Number of Sprays	Times of application weeks pre-harvest	Uninoculated	Inoculated
1. Untreated	-	-	0.7	52.1
2. Rovral	5	pf[1], pf+1[2], 5, 3, 1	0.7	22.8*
3. Rovral	3	5, 3, 1	0.4	25.7*
4. Rovral	3	6, 4, 1	0.3	24.1*
5. Rovral	3	4, 2, 1 day	0	15.6*
6. Rovral (½)	3	4, 2, 1 day	0.3	23.9*
7. Thianosan	3	5, 3, 1	0.8	45.8
8. Mildothane & Captan	3	5, 3, 1	0	47.9
9. Captan	3	5, 3, 1	2.1	41.9

PF[1] = petalfall
PF[2] = petalfall + 1 week

SED (24df) 11.0

* Significantly different from untreated ($P \leq 0.05$)

(g) **Post-Harvest Fungicide Dip 1992/93**

The percentage of Botrytis rots recorded for the inoculated treatments is shown in Table 10. Botrytis cinerea was the main rot recorded. Trace levels of Potebniamyces discolor (stalk rot) and Nectria galligena (eye rot) were also recorded. Both Ronilan and Rovral at all rates of application significantly reduced rotting compared to the untreated. Ronilan at 1ml product per litre and Rovral at 4ml product per litre gave best control of rotting. Reducing the fungicide rate of both products reduced efficacy, although not significantly so.

The addition of orchard debris to boxes resulted in the development of Botrytis rot. Dipping in water did not appear to increase the spread of Botrytis rot.

Table 10. Mean percent Botrytis rot in pear cv Conference inoculated with Botrytis and treated post-harvest with fungicide dips and stored until 11/3/93.

Fungicide Treatment	Mean % Botrytis Rot
1. Ronilan (full)	2.1*
2. Ronilan (half)	2.6*
3. Ronilan (quarter)	7.4*
4. Rovral (full)	2.0*
5. Rovral (half)	6.7*
6. Rovral (quarter)	12.2*
7. Mildothane	34.8
8. Mildothane & Captan	50.3
9. Untreated	68.8
10. Water	53.8
11. Orchard debris undipped	7.3
12. Orchard debris and water dip	5.4
SED (21 df) (treatments 1-6 and 11-12)	2.2
SED (9 df) treatments 7-10)	7.6

* significantly different from untreated ($P \leq 0.05$)

(h) **Residue Analysis 1992/93**

(i) Residue decline study for iprodione

Full details of the results of the residue analysis and analytical procedures are given in the accompanying report. A summary is given in Table 11. The residue obtained in the four experiments were similar: Levels of iprodione at 0 and 3 days after the dip treatment resulted in a lower residue than that detected at 6 weeks. Reasons for this are not clear. The residue from later sampling times were similar to those obtained at 6 weeks, indicating that the chemical residue did not decline in store. All residue levels obtained were below the MRL of 10 mg/kg fruit for pear fruit for iprodione. (UK and Codex)

(ii) Additional Residue Analysis

The results for residue analysis for iprodione and vinclozolin are given in Table 12. The residue levels detected for both Ronilan and Rovral varied considerably between replicates. Ronilan used at 1ml product per litre of water resulted in a residue of between 0.38 - 0.55mg/kg in pear fruits after six months in store, which was well below the MRL of 1mg/kg. Reducing the rate of Ronilan in the dip resulted in lower residues such that at the 0.25ml per litre rate, after six months in store, the residue of vinclozolin in pear fruits was below the level of detection. Similarly reducing the rate of Rovral in the dip tank resulted in lower residues detected in the fruit.

Rovral at the rate of 2ml product per litre applied as a programme of pre-harvest sprays with the final spray applied one week pre-harvest resulted in a lower residue than the same treatment applied as a post-harvest dip. However, when the final pre-harvest spray was applied the day before harvest, residue levels detected were very similar.

(i) **Phytotoxicity**

After two seasons of Rovral applied as pre-harvest fungicide sprays to Conference pear trees, no obvious phytotoxic effect was noted on the fruit or leaves of treated trees. Similarly no adverse reaction was detected on Conference fruits that had been dipped in Rovral.

Table 11. Chemical Residue of iprodione mg/kg in pear fruits resulting from post-harvest fungicide dip and sampled for residues at different times after treatment.

Experiment	Fungicide Treatment	Active Ingredient	Application Rate Product Per Litre-Water	Sampling Time	Iprodione Residue Mg/Kg
1	Rovral Flo	iprodione	4ml	0 days	1.49
2	Rovral Flo	iprodione	4ml	0 days	1.21
3	Rovral Flo	iprodione	4ml	0 days	1.48
4	Rovral Flo	iprodione	4ml	0 days	1.21
1	Rovral Flo	iprodione	4ml	3 days	1.61
2	Rovral Flo	iprodione	4ml	3 days	1.82
3	Rovral Flo	iprodione	4ml	3 days	1.58
4	Rovral Flo	iprodione	4ml	3 days	1.19
1	Rovral Flo	iprodione	4ml	6 weeks	2.75
2	Rovral Flo	iprodione	4ml	6 weeks	2.98
3	Rovral Flo	iprodione	4ml	6 weeks	3.43
4	Rovral Flo	iprodione	4ml	6 weeks	2.64
1	Rovral Flo	iprodione	4ml	14 weeks	2.28
2	Rovral Flo	iprodione	4ml	14 weeks	2.60
3	Rovral Flo	iprodione	4ml	14 weeks	2.72
4	Rovral Flo	iprodione	4ml	14 weeks	2.25
1	Rovral Flo	iprodione	4ml	26 weeks	3.37
2	Rovral Flo	iprodione	4ml	26 weeks	2.56
3	Rovral Flo	iprodione	4ml	26 weeks	2.80
4	Rovral Flo	iprodione	4ml	26 weeks	2.85

Table 12. Chemical residue mg a.i/kg in pear fruits resulting from pre or post-harvest fungicide treatments 1992/93 after 6 months storage in commercial pear store at 30°F (- 1.1°C)

Fungicide Treatment	Active Ingredient	Application Rate Product Per Litre Water	Replicate	Chemical Residue Active Ingredient Mg/Kg
Ronilan Fl (Dip)	vinclozolin	1ml	R1	0.55
			R2	0.38
Ronilan Fl (Dip)	vinclozolin	0.5ml	R1	0.32
			R2	0.34
Ronilan Fl (Dip)	vinclozolin	0.25ml	R1	<0.01
			R2	<0.01
Rovral Flo (Dip)	iprodione	4ml	Mean of 4 experiments	2.90
Rovral Flo (Dip)	iprodione	2ml	R1	1.05
			R2	1.13
Rovral Flo (Dip)	iprodione	1ml	R1	0.69
			R2	1.01
Rovral Flo (spray) [1]	iprodione	2ml	R1	0.93
			R2	0.74
Rovral Flo (spray) [2]	iprodione	2ml	R1	1.06
			R2	1.95

R = Replicate,

1 - 3 sprays pre-harvest applied 5, 3, 1 week pre-harvest
2 - 3 sprays pre-harvest applied 4, 2 weeks and 1 day pre-harvest

DISCUSSION:

Of the fungicides tested as alternatives to Ronilan for control of Botrytis rot, only Rovral applied as a dip gave a similar level of control. Other treatments reduced rotting compared to the untreated, but rot levels were still unacceptably high for commercial purposes. Rovral applied as a post-harvest dip was more effective in preventing contact spread of Botrytis rot than when applied as a pre-harvest spray, even if applied the day before harvest. This is most likely due to a better fungicide cover on the fruit from the post-harvest dip. It was not possible to test the effect of early orchard sprays applied at petal fall on Botrytis rot control, as very little rot developed in the uninoculated fruit. Larger scale orchard trials will be needed to evaluate this treatment in detail.

The residue levels detected for all fungicides tested were below the MRL (UK and Codex) set for these fungicides. For both Rovral and Ronilan reducing the fungicide rate resulted in lower residues in the fruit, but also appeared to reduce efficacy particularly at the lowest rate (¼ rate) used. However the technique of using innoculated fruit to test fungicide efficacy probably results in high levels of rotting, biasing the conditions in favour of the fungus. Probably under commercial conditions where inoculum levels are usually lower, the reduced rate treatments are likely to be more effective. No phytotoxicity was detected on Conference pears during the two years of the study with Rovral either as a pre-harvest fungicide spray or post harvest dip. However, tests on other pear cultivas such as Comice have not been conducted. In Belgium damage to Comice pear fruits from pre-harvest sprays has been reported (Verheyden and Creemers, 1989) although the post-harvest treatment appeared to be safe. Obviously further studies would be needed on other pear cultivars such as Comice to establish safety.

CONCLUSION:

1. Rovral (iprodione) applied as a post-harvest fungicide dip gave effective control of contact spread of Botrytis rot (Botrytis cinerea) in stored Conference pears.

2. Post-harvest dips are more effective in controlling contact spread of Botrytis rot in pears than immediate pre-harvest sprays.

3. The efficacy of fungicide sprays applied at petal fall in preventing Botrytis rot in store could not be evaluated because Botrytis failed to develop in the uninoculated fruit.

4. Residue data has been obtained for Rovral (iprodione) as a decline study following a post-harvest dip. This data combined with residue data on Rovral in pears after six months in store will be submitted for off-label approval to The Pesticides Safety Directorate (PSD).

5. Residue levels obtained for Rovral (iprodione) applied as a post-harvest dip were well below the MRL of 10mg/kg.

6. No evidence of phytotoxicity to Conference pears was noted in the two years of the study. Other pear varieties were not used in the trials. Further work is therefore necessary to check the safety of Rovral on other varieties.

ACKNOWLEDGEMENTS:

The co-operation of Mr J Edmunds and Mr Brian Checkley of J Edmunds and Son, Court Lodge Farm, East Farleigh, Maidstone, Kent, for providing the orchard trial site and the store facilities is gratefully acknowledged. Thanks for assistance are also due to ADAS research staff at ADAS, Wye. I am grateful to Martin Ridout of HRI, East Malling for statistical advice.

REFERENCES:

BERRIE, A. M. 1989. Storage rots of apple and pear in south-east England. 1980-1988 incidence and fungicide resistance. Integrated control of pome fruit diseases, 11.10BC Bulletin X11/6:229-239.

VERHEYDEN, C and CREEMERS, P 1989. Pome fruit storage disease control in Belgium: past, present and future. Integrated control of pome fruit diseases, 11.10BC Bulletin X11/6: 216/228.

DATA STORAGE:

The data will be stored in the Plant Pathology and Weed Science Department at HRI East Malling and at ADAS Wye.

APPENDIX 1

Weekly mean maximum and minimum temperatures °C and total rainfall (mm) recorded at East Malling in July - September 1991.

	TEMP °C		
Week beginning	**Max**	**Min**	**Total Rainfall (mm)**
7/7	22.3	13.1	6.8
14/7	21.6	12.6	15.8
21/7	22.3	13.2	16.8
28/7	23.7	13.5	25.9
4/8	24.6	14.8	36.8
11/8	23.7	14.3	0.8
18/8	23.0	12.5	2.9
25/8	23.4	11.1	0
1/9	23.1	13.0	Trace
8/9	21.9	8.6	Trace
15/9	21.0	9.6	10.8
22/9	17.2	9.9	18.5

APPENDIX 1

Weekly mean maximum and minimum temperatures °C and total rainfall (mm) recorded at East Malling in May - September 1992.

MEAN TEMP °C

Week beginning	Max	Min	Total Rainfall/mm
26/4	12.5	7.1	30.4
3/5	16.9	5.7	14.8
10/5	18.0	9.1	2.9
17/5	22.4	9.1	0
24/5	22.3	13.5	29.8
31/5	19.0	11.6	7.8
7/6	21.2	11.1	2.0
14/6	21.1	10.2	6.7
21/6	21.5	9.7	Trace
28/6	21.6	13.0	18.8
5/7	20.3	11.9	3.8
12/7	22.3	14.7	2.5
19/7	23.7	13.7	30.9
26/7	24.1	11.5	1.0
2/8	23.2	14.8	3.1
9/8	20.0	12.6	36.0
16/8	20.5	12.8	9.3
23/8	20.1	13.1	21.3
30/8	17.3	9.9	7.1
6/9	17.5	8.4	4.2
13/9	18.9	9.2	11.7
20/9	18.3	11.2	33.5

APPENDIX 2

Establishment of fruit temperature of 30°F (-1°C) Store 17 Court Lodge Farm, East Farleigh. 1991 from 18/9/91 - 11/10/91.

Date	Sensor 1	Sensor 2	Sensor 3	Sensor 4	°F Mean Store Temp
18/9 (Store loaded)	52	54	53	51.5	52.6
19/9	51	52.5	53	53	52.4
20/9	47.5	50	52.0	52.5	50.2
21/9	43.5	46.5	51	51	48
22/9	41.5	43.5	48	48	45.3
23/9	38.5	41	46	46	43
24/9	35.5	38	42.5	42.5	40
25/9	34.2	36.2	40.5	40.5	38
26/9	32.2	34.8	39.5	39	36
27/9	30.8	33	37.5	37.5	35
28/9	31	31.5	35.5	36	34
29/9	29.5	29.5	34.5	34.5	32
30/9	29	29.5	33	33.2	31
1/10	29	29	32	32.2	30
2/10	30	30	31.8	32	31
3/10	29	29	31.5	32	30
4/10	29	29	31	31.8	30
5/10	30.2	30	31.2	32	31
6/10	29	29.5	31.5	31.5	30
7/10	29.5	29.5	31	31.8	30
8/10	29	29	31	31.5	30
9/10	29	29	31	31.2	30
10/10	29	29	31	31.2	30
11/10	29	29	30	31	30

APPENDIX 2

Maximum, minimum and mean temperatures recorded in store 17, Court Lodge Farm, East Farleigh, during pear storage in 1991/92.

Month	Minimum temp °F	Maximum temp °F	Mean temp °F *
October	28.0	31.5	30.0
November	28.0	31.8	30.0
December	28.0	32.0	29.5
January	28.0	32.0	30.0
February			

* Mean of 4 temperature sensors

APPENDIX 2:

Establishment of temperature of 30°F (-1°C) store 12, Court Lodge Farm, East Farleigh 1992.

Store loaded on 10.9.92.

Unfortunately temperature records for store 12 were mislaid during the sale of the farm in 1993 and are not available for inclusion in this report. The store manager notes that the recommended fruit temperature of 30°F (-1°C) was achieved more rapidly in 1992 than the previous year and nearer the recommended total time of 10 days. There were no problems with store 12 during the storage period from 10.9.92 to 11.3.93.

APPENDIX 2:

Maximum, minimum and mean temperatures recorded in store 12, Court Lodge Farm, East Farleigh, during pear storage in 1992/93.

Data not available for inclusion in the report due to the store records being mislaid during the sale of the farm.

APPENDIX 3

EXPERIMENT PROPOSAL/PROTOCOL
1991 and 1992

EXPERIMENT PROPOSAL TO APRC

1. Title of Project

Botrytis Rot of Pear: Evaluation of alternative fungicides to Ronilan for control of botrytis rot in stored pears, applied as pre-harvest sprays or as a post-harvest drench.

2. Background

Botrytis rot of stored pears caused by the fungus Botrytis cinerea is consistently the most important cause of rotting in stored pears. (Berrie, 1989). Before the introduction of post-harvest treatments losses due to botrytis were considerable and restricted the storage life of pears to December/January. The introduction of benzimidazole fungicides as post-harvest treatments (eg. Benlate, Bavistin) enabled the potential storage life to be extended to April/May thus increasing the financial returns to growers. Unfortunately Botrytis cinerea readily developed resistance to the benzimidazole fungicides and within 2-3 years the efficiency of the post-harvest drench was much reduced. Studies showed that up to 75 per cent of Botrytis cinerea isolates from stored pears were resistant to benzimidazole fungicides (Berrie, 1989). However, this treatment still gave good control of brown rot (Monilinia fructigena) which can occasionally be responsible for serious losses in store.

Trials with vinclozolin (Ronilan) in the early 1980's demonstrated the effectiveness of this product in controlling Botrytis rot (including those isolates resistant to benzimidazole fungicides) brown rot and suppressing penicillium rot. Since its commercial introduction, rotting in stored pears has been maintained below two per cent losses (Berrie, 1989). Almost all pears and especially those destined for long term store were routinely treated with

Ronilan as a post-harvest treatment. Unfortunately even when the product was applied according to the label recommendation and good agricultural practice, this did not guarantee that the residue was below the Maxim Residue Level set. Therefore in September 1990 the Company, BASF withdrew their support for the post-harvest recommendation on apples and pears. Since then new toxicological studies have revealed other problems and the use of Ronilan has become severely restricted in Agriculture/Horticulture. Therefore there is now no effective treatment for control of botrytis rot in stored pears.

Botrytis cinerea is a ubiquitous fungus affecting a wide range of crops with no evidence of host specialisation. Unlike brown rot, botrytis rot is rarely, if ever, seen on fruit in the orchard prior to harvest although it commonly occurs as a saprophyte on rotting debris on the orchard floor. It appears in store, forming nests of rots if uncontrolled. The fungus usually enters the pear through wounds which probably occur during harvest and which may only be minute blemishes in the skin. Occasionally the fungus can spread into the pear from stalk end infection or from calyx end infections, but the usual entry point is on the cheek of the fruit via wounds. One main source of inoculum is debris, such as leaf, twig and soil picked up on bulk bins during harvest or debris remaining in the bin from the previous season. Drenching an ineffective fungicides can actually increase the risk of rotting by spreading inoculum in the drenching solution. While attention to hygiene, particularly cleaning out bulk bins can reduce the risk to some extent, present harvest techniques make it impossible to avoid the introduction of leaves and other tree debris into the bin.

Because of the nature of the disease post-harvest treatments are likely to be most effective in controlling the rot in store. However, the long term future of such treatments is doubtful. Therefore any investigation on alternative

chemical treatments to Ronilan must also examine their effectiveness as pre-harvest treatments as well as post harvest drenches.

3. Potential Benefits

The importance of Botrytis cinerea as a cause of losses in stored pears has already been stated. The alternative post-harvest treatments at present available are Captan and the benzimidazole fungicides such as Benlate. The latter group of fungicides will be of limited effectiveness because of fungicide resistance. The latter, to be of any use, would need to be applied at the full rate with likely problem of visible chemical deposit on the fruit. The effectiveness of pre-harvest fungicide sprays in controlling botrytis rot is unknown.

The study would therefore benefit the industry by investigating a possible alternative treatment to Ronilan, and by assessing the efficacy of pre-harvest sprays for controlling botrytis.

4. Objective

- To identify an alternative fungicide treatment to Ronilan for control of botrytis rot on stored pears.

- To obtain the necessary residue data to enable any effective treatment to receive Off-Label Approval where necessary, for use on pears either as pre-harvest or post-harvest treatments.

- To assess the efficacy of pre-harvest fungicide sprays in controlling botrytis rot on pears.

5. Description of proposed work

The investigation would be in two parts - pre-harvest sprays and post-harvest dips/drench. The following chemicals would be tested, with Ronilan (vinclozolin) included as standard.

Product	Active Ingredient
Ronilan	vinclozolin
Rovral	iprodione
Mildothane	thiophanate-methyl
Elvaron	dichlofluanid
Captan	captan
Unicrop Thianosan	thiram
Fungaflor	imazalil
Untreated	-

(a) Pre-harvest sprays

Sprays would be applied high volume to pear tree, cv Conference in August and/or September by hand-held applicator. A two-spray programme is planned. Each treatment would be replicated four times. An assessment of spray cover on the fruit would be made in the orchard.

At the recommended harvest time a 30 lb unit of pears (approx. 100-120 fruits) would be picked from each plot and cold stored under commercial conditions until the following February/March. At this time an assessment of rotting due to botrytis and other fungi would be made for each plot.

In addition, at harvest, a further 30 lb unit of pears would be picked from each plot. Ten pears inoculated with Botrytis cinerea (as described below) would be placed at random in each 30 lb unit. These would be similarly stored and assessed for rot spread the following February/March.

At harvest and again post-store, samples of fruit would be taken from each plot for residue analysis, where necessary.

(b) Post-harvest dips/drenches

Pear fruit, cv Conference, would be inoculated with an isolate of Botrytis cinerea by inserting mycelium below the skin at two points on the fruit and sealing with coloured tape. The fruit would be left for several days at room temperature to allow rot development. Ten inoculated fruit would then be placed at random among healthy fruit (100-120 fruit) in each 30 lb unit used. Such boxes would then be dipped in the test fungicides listed above and placed in cold-store under commercial conditions until the following February/March. Each treatment would be replicated four times. On removal from store the spread of the botrytis rot would be assessed.

Samples for residue analysis where appropriate would be taken immediately post-treatment and again post-store.

6. Starting date and duration

August 1991 for two years initially.

7. Staff responsibilities

Project Leader: Dr A Berrie,

Plant Pathologist, ADAS

Wye Advisory Centre

Olantigh Road

Wye, Ashford, Kent TN25 5EL

8. Location

ADAS Laboratories at Wye.

An orchard in Kent.

9. Costs		1991	1992*
Salaries		6007	
Materials and incidental costs	+	1205	
Residue Analysis		4000	
Total		£11,212.00	

* Costs will depend upon results of first year's work

+ Includes possible compensation for loss of fruit.

Some of this cost may not be incurred

PEAR: Evaluation of alternative fungicides to Ronilan applied as pre-harvest sprays or as a post harvest dip for control of Botrytis rot in stored pears 1992/93.

Objectives

Following the results of the 1991/92 experiment, which identified Rovral (iprodione) as the most promising alternative to Ronilan (vinclozolin), the following objectives were included for 1992/93.

1 To examine the effects of early sprays applied at petal fall on control of botrytis rot.

2 To examine the effects of orchard sprays applied nearer to harvest on control of botrytis rot.

3 To assess the efficacy of lower rates of iprodione (Rovral) and vinclozolin (Ronilan) applied as post-harvest dips on control of botrytis rot.

4 To assess the efficacy of half rate thiophanate methyl (Mildothane) in combination with half rate captan applied as pre harvest sprays or a post harvest dip in controlling botrytis rot.

Methods

Methods for pre-harvest sprays and post-harvest dips were as in 1991/92. Treatments applied are shown in Tables 5 and 6. Residue analysis is concentrating on obtaining an off-label approval for Rovral (iprodione) as a post harvest treatment.

TABLE 6 Fungicide treatments applied as a single post-harvest dip 1992/93

Treatment	Product	Active Ingredient	Product rate/ 1000 l
1	Ronilan Fl	Vinclozolin	1000 ml
2	Ronilan Fl	"	500 ml
3	Ronilan Fl	"	250 ml
4	Rovral Fl	iprodione	4000 ml
5	Rovral Fl	"	2000 ml
6	Rovral Fl	"	1000 ml
7	Mildothane	thiophanate-methyl	2000 ml
8	Mildothane + Captan	thiophanate-methyl + Captan	1000 ml + 600 g
9	Untreated	-	-
10	Water	-	-
11	Pears + orchard debris undipped	-	-
12	Pears + orchard debris + water dip.	-	-

TABLE 5 Fungicide treatments and rates of application as pre-harvest sprays applied to run-off 1992/93.

Treatment	Product	Active ingredient	Product rate/ litre water	No of sprays	Times of application (weeks pre-harvest)
1	untreated	-	-	-	-
2	Rovral Flo	iprodione	2 ml	5	pf, pf + 1, 5,3,1
3	Rovral Flo	iprodione	2 ml	3	5, 3, 1
4	Rovral Flo	iprodione	2 ml	3	6, 4, 1
5	Rovral Flo	iprodione	2 ml	3	4, 2, 1 day
6	Rovral Flo	iprodione	1 ml	3	4, 2, 1 day
7	Thianosan	thiram	2 g	3	5, 3, 1
8	Mildothane + Captan	thiophanate + Captan	1 ml + 0.825 g	3	5, 3, 1
9	Captan	Captan	0.825 g	3	5, 3, 1

pf = petal fall