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Final Contract Report for

Apple and Pear Research Council

funded-project (SP118) on

Investigation on resistance of apple powdery mildew to fungicides

Final Report (March 2001)

Investigation on resistance of apple powdery mildew to fungicides

Report to: Apple and Pear Research Council

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Summary

The Apple and Pear Research Council have funded a three-year project at HRI-East Malling, which started in April 1998, to investigate the extent to which apple powdery mildew strains resistant to fungicides have developed. The research work is based mainly on monitoring disease development, after application of a range of commercial apple mildew fungicides, in orchards where control of mildew is difficult.

Six commercially available fungicides were tested: Rubigan (fenarimol), Systhane (myclobutanil), Topas (penconazole), Bayleton (triadimefon), Nimrod (buprimate), and Dorado (pyrifenox). The following conclusions are drawn from this study:

1. All six fungicides significantly reduced the incidence of mildew, compared to untreated control.
2. There were significant differences in the efficacy against mildew between the six fungicides. Of the six fungicides tested, Rubigan and Bayleton were least effective.
3. Application interval has considerable impacts on mildew control, even more than the application rate. Longer intervals resulted in significantly more mildew that shorter intervals.
4. Of the fungicides tested, there is considerable evidence suggesting that there are mildew strains present in the experimental orchards that are tolerant to Dorado, especially when applied at an interval of 14 days. Evidence for reduced sensitivity to Systhane is less clear, compared to that for Dorado.
5. Comparisons of the efficacy of Dorado and Systhane against mildew with the results from other trials suggests that isolates tolerant to Dorado and to a lesser degree Systhane are currently very localised.
6. The importance of application interval indicates that it is essential to maintain a good coverage of the emerging leaves for achieving good mildew control, which may also suggest low volume applications may also significantly reduce the control efficacy.

Introduction

The main UK apple cultivars Cox and Gala (dessert), and Bramley's Seedling (culinary) are susceptible to a range of fungal diseases which reduce yield and quality, and result in further losses post-harvest through rotting in store. The market requirement for high quality blemish-free fruit, and the difficulties of achieving control once the diseases have become established in the orchard, have led to the application of routine fungicide sprays, applied from bud-burst to harvest, at 7-14 day intervals. Such routine programmes, though costly, have usually proved successful, reliable and simple for farm managers to operate.

Most of the routine fungicide programme is devoted to the control of apple scab *(Venturia inaequalis)* and powdery mildew *(Podosphaera leucotricha)*. However, even such intensive programmes, can still result in poor control of mildew. This can be due to a number of reasons such as poor spray cover or failure to adjust the spray programme in response to increased mildew risk However, often such failure is attributed to the development of fungicide-resistant mildew strains. Such claims are not without some basis since control of apple mildew is heavily dependent on the DMI fungicide group (e.g. Systhane, Topas, Dorado, Rubigan) and the gradual development of strains of powdery mildew which are insensitive to the DMI fungicides has been reported in other crops, such as grape and barley. The existence of such resistance will have significant impact on the control of apple powdery mildew. However, no critical studies have been conducted in apple to investigate the extent to which mildew strains resistant to fungicides have developed. The Apple and Pear Research Council have funded a three-year project at HRI-East Malling, which started on April 1998, to address this problem.

The research work is based mainly on monitoring disease development, after application of a range of commercial apple mildew fungicides, in orchards where control of mildew is difficult. The research work was conducted in a period of three years (April 1998 - March 2001). Results from the first two years have already been reported to APRC and presented in the APRC newsletter. This final report is mainly focussed on the results from the last year and the general conclusion from this project.

April-1998 - March 1999

Materials and methods

Four orchards all located in East Kent with a history of poor mildew control were selected for field trials; two were growers advised by FAST, and two were growers advised by ADAS and HRI.

In each orchard, 24 trees were selected at random, allocated randomly to one of the eight treatments: six fungicides (Systhane, Bayleton, Nimrod, Topas, Dorado and Rubigan), water and untreated, giving three trees per treatment. In late May, on each tree, five shoots with primary mildews and five extension shoots free of mildews were selected and tagged. Fungicides at the full label-recommended dose or water were then applied to selected shoots with a hand-held sprayer to run-off. About two weeks later, mildew sporulation was assessed on these treated or untreated primary mildew. About three weeks later, the number of secondary mildew lesions on those treated or untreated extension shoots were assessed.

In early July, the experiments on extension shoots were repeated again.

Results

Results on the anti-sporulant efficacy of the fungicides showed there were small but significant differences between the performance of the fungicides at the four farms. The six fungicides significantly reduced the sporulation of primary mildew two weeks after treatment. Of the six fungicides, Systhane and Bayleton were the best, Dorado and Topas were the next best, and Nimrod and Rubigan were the least effective. No significant problems of mildew resistance to fungicides were evident.

For the mid-May test, the overall difference in the treatments was small but significant. All the fungicides significantly reduced the numbers of lesions compared with the untreated leaves. Of the six fungicides, Systhane and Nimrod were the worst while the other four were similar in effect. Generally, there were only small differences in the effects of the fungicides between the four farms. The effects of Bayleton, Dorado, Topas and Rubigan were consistent among the four farms. Systhane had significantly less effect on one farm (farm 2) than at the other three; Nimrod also had significantly less effect at one farm (farm 4) than at the other three.

For the early-July test, there were large differences between the farms. On those where leaves were treated, the numbers of lesions recorded on farms 1 & 2 were at least twice those on farms 3 & 4. The six fungicides significantly reduced the numbers of lesions compared with the untreated leaves. Systhane was the least effective, even though the fungicide was the best of the six in an unrelated work conducted recently at East Malling. For the other five fungicides, the order from the most to the least effective was Dorado, Bayleton, Topas, Nimrod and Rubigan. There were large and significant differences in the effects of fungicides at the four farms; the effect of only Dorado was consistent among the four farms. Systhane, Rubigan and Topas had significantly less effect at farms 1 and 2 than at the other farm, and were less effective at farm 4 than at farm 3. Bayleton performed very badly at farms 1 and 4. Nimrod was significantly less effective at farm 2 than at the other three.

The full rate used is based on the run-off rate recommended by the manufacturer and is shown in Table 1 for the six fungicides.

Table 1. The full rate used for the six fungicides tested.

Fungicides*	Rate (ml/ltre)
Nimrod	0.35
Topas	0.18
Systhane	0.115
Dorado	0.1
Rubigan	2
Bayleton	0.1

*: All fungicides excerpt Nimrod belong to the DMI group; Dorado and Rubigan are in slightly different groups to other three DMI fungicides.

April 1999 - March 2000

Materials and methods

Two orchards located in East Kent were selected from the four orchards used for the previous year's work (farms 1 and 2).

In each orchard, 39 trees were selected at random, and allocated to one of the 12 treatments: six fungicides (Systhane, Bayleton, Nimrod, Topas, Dorado and Rubigan) at two application intervals (7 and 14 days) and untreated, giving three trees per treatment. In late May, on each tree, five extension shoots without mildew were selected and tagged. Fungicides at the full label-recommended dose were then applied to those selected shoots with a hand-held sprayer to run-off at an interval of either 7 or 14 days until mid-July when shoots stopped growing. The numbers of secondary mildew lesions on the extension shoots were assessed weekly.

Results

The results showed clearly that there were significant differences between the severity of powdery mildew on the two farms. The incidence of mildew on farm 1 was about 3 times that recorded on farm 2, with an average of six lesions per leaf. One possible reason for this significant difference is the phytotoxicity of fungicides. Many treated shoots were stunted on the trees on farm 2 and they therefore appeared to be less tolerant of the fungicides at high doses compared to the trees on farm 1. However, the overall efficacy of fungicides and the effects of the spray intervals were consistent between the two farms.

All six fungicides significantly reduced the mildew severity compared with the untreated. The six fungicides can be classified into three groups based on their efficacies, i.e. 1) Systhane (best), 2) Dorado, Nimrod and Topas (moderate), and 3) Bayleton and Rubigan (worst). On average, there were 0.1, 0.3 and 1 lesion per leaf for the three groups, respectively, compared with 4 lesions per leaf on the untreated shoots. Applying fungicides at 14 days resulted in nearly three times more disease than a spray interval of 7 days. Only Systhane appeared equally effective at both intervals with no significant difference in mildew severity between the two timings.

April 2000 - March 2001

Materials and methods

This year, the work has concentrated on the effects of varying the fungicide dose on the efficacy of the fungicides; only Systhane, Nimrod, Topas and Dorado were included in these studies.

In each of two orchards, 51 trees were selected at random, and allocated to one of the 17 treatments. These included four fungicides (Systhane, Nimrod, Topas and Dorado), at two application intervals (7 and 14 days) and two application doses (half and full rates of the 7-day label rate), and untreated, giving three trees per treatment. In late May, on each tree, five extension shoots without mildew were selected and tagged. Fungicides at the appropriate rate were then applied to run-off to the selected shoots with a hand-held sprayer at 7 or 14 day intervals until the end of shoot growth in mid-July. The number of secondary mildew lesions on the treated or untreated extension shoots was assessed weekly.

A large field trial was also conducted in Suffolk to determine the effect of spray dose on mildew control on Cox. There were four treatments: ¼ and full rates of Systhane at 7 day interval, standard farm program and untreated (i.e. no mildew fungicides applied). Apart from the untreated, there were two replicates for each treatment; each plot had four rows of trees, about 70-100 trees within each row. In late May, on each of 20 chosen trees in each plot, five extension shoots without mildew were randomly selected and tagged. Fungicides at the appropriate rate were applied to the whole plot using the standard orchard air blast sprayer at 7 day intervals until the end of shoot growth in mid-July. Four assessments were made. In the first three assessments, the number of leaves with mildew lesions on the top five youngest fully extended leaves on each shoot was recorded; for the last assessment, the number of mildew lesions on each of the top five youngest fully extended leaves on each shoot was counted. In addition, the overall incidence of primary mildew for the orchard was estimated in May.

Results

In total, there were 8 assessments made; however, the first and the last assessments were excluded from the analysis since these two assessments were most likely to be affected by factors other than the treatments.

Analysis of variance shows that differences in the number of mildew lesions between the two farms were very small and only significant for the assessment on 5/6; the data (number of lesions) were logrithm-transformed before analysis of variance. Interactions between farm and treatment were significant for all six assessments but accounted for far less variation than that by the main treatment effects (Table 2). Therefore, the data were pooled over the two farms.

Most of the 16 fungicide treatments had significantly reduced the number of mildew lesions compared to the untreated control. There were only a few treatments where there were no significant reductions (Table 3), most of which were associated with Dorado.

Of all the treatment factors (Fungicide, Rate, Application interval and their interactions), most of treatment variation (about 70-80%) was consistently due to the main effects of fungicides and application intervals (Table 2). Application of fungicides at an interval of 14 days resulted in significantly more mildew than application at an interval of 7 days. The effect of rate was significant but small. All the interactions except interval x rate were generally significant but small and not consistent over the six assessments (Table 2).

Table 4 shows the average number of mildew lesions per leaf for each combination of fungicides and application intervals. Of the four fungicides, Dorado was the least effective against powdery mildew and Nimrod was most effective. Topas, when applied at 7-day-intervals, was as effective as Nimrod, but not at 14-days-intervals. The differences between Systhane and Topas were generally small. Dorado was still effective, reducing mildew by *c. 50%* when applied at 7-days-intervals; however, when applied at an interval of 14 days, there were no reductions in powdery mildew for three of the six assessments, compared to the untreated control.

Table 2. Percentage of total treatment variation accounted for by each term (from analysis of variance table)

Terms	05-Jun	10-Jun	23-Jun	29-Jun	05-Jul	19-Jul
Farm	10	0	1	0	3	3
Treatment	77	89	69	81	76	80
Farm.treatment	13	11	30	19	21	17
Fungicide	46	45	53	38	32	68
Rate	0	5	3	8	1	5
Interval	17	22	22	48	54	14
Fungicde.rate	15	6	4	2	5	10
Fungicide.interval	7	4	13	1	5	0
Rate.interval	0	0	2	1	1	1
Fungicide.interval.rate	15	18	3	2	2	2

Table 3. Average number of mildew lesions per leaf at each assessment date for each treatment over two farms.

Fungicide	Application Interval	Rate	Assessment dates					
			05-Jun	10-Jun	23-Jun	29-Jun	05-Jul	19-Jul
Nimrod	7	Full	0.16	0.12	0.08	0.06	0.17	0.22
Nimrod	14	Full	0.17	0.24	0.35	0.45	0.52	0.52
Nimrod	7	Half	0.16	0.06	0.07	0.06	0.18	0.17
Nimrod	14	Half	0.23	0.18	0.24	0.54	0.35	0.47
Topas	7	Full	0.14	0.01	0.05	0.02	0.01	0.04
Topas	14	Full	0.26	0.45	0.75	0.28	0.21	0.28
Topas	7	Half	0.19	0.08	0.08	0.10	0.02	0.04
Topas	14	Half	0.49	0.37	0.78	0.60	0.66	0.34
Systhane	7	Full	0.31	0.50	0.06	0.12	0.05	0.08
Systhane	14	Full	0.36	0.36	0.74	0.50	0.45	0.79
Systhane	7	Half	0.22	0.24	0.24	0.44	0.17	0.30
Systhane	14	Half	0.86	1.15	0.86	***0.72****	***0.62***	0.42
Dorado	7	Full	0.58	0.15	0.74	0.54	0.36	0.38
Dorado	14	Full	***1.76***	0.79	0.90	***0.83***	***1.14***	0.69
Dorado	7	Half	0.61	0.80	***1.90***	0.43	0.31	1.01
Dorado	14	Half	0.66	0.81	0.85	***1.32***	***1.01***	***1.75***
Control			1.53	1.79	1.45	0.94	0.68	1.25

*: numbers in bold italics are not significantly different from the untreated control.

Table 4. Average number of mildew lesions per leaf at each assessment date for each combination of fungicide and application interval over two rates and two farms.

	05-Jun	10-Jun	23-Jun	29-Jun	05-Jul	19-Jul
7 days						
Nimrod	0.16	0.09	0.08	0.06	0.17	0.19
Dorado	0.59	0.46	1.32	0.48	0.34	0.68
Systhane	0.27	0.37	0.15	0.28	0.11	0.19
Topas	0.17	0.05	0.07	0.06	0.01	0.04
14 days						
Nimrod	0.20	0.21	0.29	0.49	0.45	0.49
Dorado	1.23	0.80	0.88	1.09	1.07	1.25
Systhane	0.61	0.76	0.80	0.60	0.54	0.59
Topas	0.38	0.41	0.76	0.45	0.44	0.31
Control	1.53	1.79	1.45	0.94	0.68	1.25

Results from the field trials in Suffolk are summarised in Table 5. Powdery mildew in the orchard was very severe. In the untreated plots, about 94% of leaves were infected with mildew by August 1st. Applying Systhane at 25% rate reduced the disease incidence by nearly half; applying Systhane at the full rate further reduced the incidence by nearly 20%, though the reduction in the average number of lesions per leaf was very small. The efficacy of the full rate programme was slightly worse than the standard farm programme. The results clearly demonstrated the risk of cutting down the application rate.

Table 5. Effects of fungicide dose on mildew control on apple cv Cox (trials conducted in Suffolk in 2000).

Treatment	% mildewed leaves					Mildew lesions per leaf on 1/8
	2/6	16/6	30/6	14/7	1/8	
Untreated	2.9	15.6	39.0	62.2	94.4	2.8
25% Systhane	0.3	24.2	23.0	40.8	47.7	1.2
100% Systhane	0.1	21.4	20.2	28.6	28.2	1.0
Farm programme	0.4	18.0	29.8	43.2	19.1	0.6

Other minor works

Preliminary studies were carried out on *in vitro* storage of mildew lesions. Results indicated that conidia of powdery mildew on detached leaves *in vitro* remained viable for up to five weeks at 5°C and for four weeks at 10°C. Further studies are needed to develop protocols for testing fungicide sensitivity *in vitro*.

A questionnaire on the problems of controlling apple powdery mildew was produced and sent with the April APRC newsletter to APRC members. Unfortunately, there were only a few replies, and consequently no meaningful analysis can be done.

General conclusions

1. The results to date suggest that there is no evidence of a sudden change in the sensitivity of mildew to the fungicides tested. However, it does appear that both the efficacy and persistence differed significantly between the fungicides. The fungicides in the experiments were all applied at the full-rate to run-off. Thus, the differences in the results are unlikely to be due to differences in spray cover. Obviously successful mildew control is dependent on a number of factors that

include a good understanding of the epidemiology of the fungus as well as recognising the limitations of the fungicides available for control.

2. Of the fungicides tested, there is considerable evidence suggesting that there are mildew strains present in the experimental orchards that are tolerant to Dorado, especially when applied at an interval of 14 days. Evidence for reduced sensitivity to Systhane is less clear, compared to that for Dorado. In related work, the persistence of Dorado, when applied at quarter rate was significantly, reduced to 3-6 days compared to other fungicides. Therefore, care should be taken when using Dorado in controlling apple mildew.

3. Related work on understanding the mode of actions of mildew fungicides has been recently conducted at East Malling. In these tests, Dorado and Systhane were shown to be as effective as Nimrod and Topas in controlling mildew either as a protectant or a curative treatment or an anti-sporulant. Compared to the loss of effectiveness of Dorado in the test orchards, it suggests that isolates tolerant to Dorado and to a lesser degree Systhane are currently localised.

4. The results clearly showed that application interval has considerable impacts on mildew control, even more than the application rate. Applying fungicides at 14 days resulted in two to three times more disease than a spray interval of 7 days. The results may suggest that the translocation/spread of fungicides within a leaf is very limited. Thus, maintaining a good coverage of the emerging leaves is paramount important in achieving good mildew control, this may also suggest low volume applications may also significantly reduce the control efficacy.