Report prepared for the
Horticultural Development Council

Tomato: Fungicide sensitivity testing and comparison of fungicides for control of powdery mildew

(1992-1993)

PC26a

Final Report (February 1994)

Project Number:	PC26a
Title:	Fungicide sensitivity testing and comparison of fungicides for control of tomato powdery mildew.
Project Leader:	Dr T.M. O'Neill
Location of Project:	ADAS Cambridge ADAS Reading
Project Co-ordinator:	Dr N.O. Dungey
Date project commenced:	December 1991
Date completion due	March 1994
Key words:	Tomato, powdery mildew, *Erysiphe* sp. fungicides, resistance

CONTENTS

APPLICATION

The objective of the project is to provide information on the use of fungicides for control of tomato powdery mildew. Treatments and timings have been identified which provide good control of the disease and do not affect plant growth. Progress has been made on developing a technique for testing mildew samples for fungicide resistance. A range of sensitivities to fenarimol (Rubigan) was found among samples from commercial crops and growers should not rely solely on this product to control powdery mildew. There is opportunity for growers to achieve good control of mildew by careful product choice and appropriate timing of applications.

SUMMARY OF RESULTS

The objectives of the work were: (1) to examine the sensitivity of young tomato plants to fungicide treatment during the winter (2), to examine the effectiveness of a range of fungicides when mildew has just established in a crop and also under conditions of heavy and prolonged disease pressure (3), to devise a method for testing isolates of mildew for resistance to Benlate, Nimrod and Rubigan, and to test isolates from commercial crops.

When young tomato plants were sprayed twice with Benlate, Elvaron, Nimrod, Repulse, Rubigan or Thiovit plus Agral (a non-ionic adjuvant) at up to twice the label or specific off-label rate, there was no effect on plant growth or number of flowers and none of the products caused leaf scorch or distortion. Repulse left a very noticeable spray deposit even at half the recommended spray rate. Rubigan and Nimrod left virtually no visible spray deposit while other products were intermediate.

A single spray of Elvaron, Nimrod, Repulse, Rubigan or Thiovit plus Agral was applied when 20% of tomato plants showed trace levels of mildew or 3 weeks later when all plants showed low levels of infection (1-2% leaf area). All products at either timing gave good control of the disease and there were no significant differences between products. Disease control persisted for approximately 6 weeks. When sprays were applied at both timings levels of mildew were kept very low. Spraying plants twice-weekly with water did not control or aggravate development of mildew.

The same products applied once as single sprays when plants showed approximately 10% leaf area affected by mildew resulted in moderately good disease control. Four weeks after treatment the level of mildew on plants treated with Rubigan was significantly less than on plants treated with Nimrod.

The sensitivity of samples of powdery mildew collected from commercial crops to three fungicides was determined using detached leaves and young plants. A range of sensitivities to Rubigan was found. One sample, from a Rubigan-treated crop, grew on leaves treated with Rubigan at $^1/_{10}$ the label recommended spraying rate. A sample tested against Benlate, Nimrod and Rubigan was sensitive to Benlate and less sensitive to Nimrod and Rubigan at the concentrations tested. Possible reasons for some samples failing to establish on detached leaves were investigated. A sample inoculated on to young plants established well and both Nimrod and Rubigan gave good control at $^1/_{10}$ of the label recommended rates. In a spore germination test, the frequency of germination declined as Rubigan concentrations increased although most samples showed a low germination on agar amended with full-rate fungicide. Further tests are required to determine if poor disease control in Rubigan-treated crops is associated with the presence of mildew showing reduced sensitivity to Rubigan.

ACTION POINTS FOR GROWERS

1. Growers should apply fungicide treatment as soon as possible after mildew is found. Early application should reduce the disease to a very low level, and there is a wide range of products which can be used successfully at this time.

2. Rubigan appears to be the best product if mildew is severely affecting a crop. It has a persistant effect and leaves only a little deposit. Thiovit plus Agral appeared to be only slightly less effective, although it left more spray deposit.

3. Because of the risk of tomato powdery mildew developing resistance to Rubigan, growers should also use alternative products (eg Thiovit) in a spray programme.

4. Thiovit is permitted in organic crops grown to UKROFS standards. For production to Soil Association standards a grower will require permission from the certification committee before making routine use of sulphur (Thiovit). The wetter Agral is not approved by UKROFS and use of sulphur without a wetter may reduce its efficacy against mildew.

INTRODUCTION

Powdery mildew (*Erysiphe* sp.) first occurred on protected tomatoes in England in 1987 (Fletcher *et al.*, 1988). It is now widespread in the country and many growers must resort to fungicide spraying to prevent it causing significant yield loss and reduced fruit quality. Experience is showing that outbreaks of the disease may occur at any time from propagation onwards and may occur more than once on the same nursery during a season. Consequently some growers need to apply several sprays. Unfortunately, there are no reliable alternatives to fungicide spraying for control of tomato mildew. Earlier work (PC26 and MAFF-funded work) indicated that fenarimol (Rubigan), bupirimate (Nimrod), dichlofluanid (Elvaron), chlorothalonil (eg Bravo 500, Repulse) and benomyl (Benlate), which are all MAFF approved for use on tomatoes, can give useful protection. Grower experiments indicated that sulphur (Thiovit) was also effective. It was uncertain how well these fungicides would perform under prolonged or heavy disease pressure.

There is some evidence that spraying with water alone can control some powdery mildews (Yarwood, 1939). The effect of water on development of tomato powdery mildew was unknown.

Fenarimol, bupirimate and benomyl all have a very specific (single site) mode of action which is likely to result in resistance problems if they are used intensively. Resistance to all three fungicide groups has been reported in cucumber powdery mildew (*Sphaerotheca fuliginea*) (Schroeder & Provvidenti, 1968; Bent *et al.*, 1971; Schepers, 1983). Chlorothalonil, dichlofluanid and sulphur are all multi-site fungicides and there is less risk of powdery mildew developing resistance to these types of fungicide. Monitoring fungicide sensitivity in order to detect early development of resistant strains is not simple because powdery mildew is an obligate parasite and cannot be grown on artificial media. Grower experience in 1988 and 1989 indicated that 2 or 3 well-timed sprays were often sufficient to eliminate mildew. In 1991 growers and advisers reported that some fungicides were not providing adequate control.

When outbreaks of powdery mildew occur during the winter there are fears that spraying with Nimrod, Rubigan or Thiovit may cause damage to the soft growth on young plants. These products are permitted as Specific Off-Label Approval (SOLA) uses and such use is at growers' own risk.

MATERIALS AND METHODS

Phytotoxicity tests

Plants of cvs. Calypso and Pronto were grown in rockwool cubes at ADAS temperatures (T.A. Wright, pers. comm.) and with supplementary lighting (50,000 lux) provided from late December. No supplementary CO_2 was given.

Fungicide treatments were applied on 8 January 1992, when plants had 7 true leaves more than 2 cm long, and again on 23 January, when plants had 12 true leaves more than 2 cm long. Fungicides were applied to the point of run-off using a knapsack sprayer with a flat-fan nozzle. Products were applied at the label recommended rate for tomatoes or according to the Specific Off-Label Approval (SOLA). Each product was also applied at x2 and x ½ these rates, and there were also water-sprayed and unsprayed controls. Rates of application are summarised in the table below.

Product	Active ingredient	Rate of application/100 l		
		x2	x1	x½
Benlate	benomyl	200g	100g	50g
Elvaron	dichlofluanid	200g	100g	50g
Repulse	chlorothalonil	440 ml	220 ml	110 ml
Rubigan	fenarimol	36 ml	18 ml	9 ml
Nimrod	bupirimate	200 ml	100 ml	50 ml
Thiovit*	sulphur	400g	200g	100g

*+ 6ml Agral/100 l

Plants were assessed for leaf size, flower number and spray deposit on 6 February 1992, 14 days after the second spray.

Fungicide Trials

Trial 1

This was in a crop of cv. Counter in a single-span glasshouse (12x8x4 m) at ADAS Reading. The layout was a randomised block design with three replicates A plots consisted of four plants, with an unsprayed guard of two plants between plots. Plants were set out on rockwool slabs on 27 April. Powdery mildew was observed in the crop on 11 May, prior to introduction of infector plants. Five plants affected by powdery mildew were set out in each pathway the following day. Treatments were applied at the product label or SOLA rate. The control treatment consisted of plants left unsprayed. In an

additional treatment, plants were sprayed with water twice-weekly. Fungicides were applied 5 days after the first sign of mildew in the crop (16 May) or 17 days later (2 June), or on both dates. Sprays were applied to run-off using a knapsack handpump sprayer with a 110° flat fan nozzle. The leaf area affected by mildew on six leaves per plant (two each in the lower, middle and upper canopies) was assessed weekly for 8 weeks from 15 May to 9 July.

Trial 2

The crop described above was layered on 10 July and plots were re-allocated. There were three replicates of treatments arranged in randomised blocks. Plots consisted of 6 plants with a guard of 2-6 plants between plots. Two double rows of the crop, between treated rows, were left unlayered and unsprayed to provide a high disease pressure for the duration of the trial. An extractor fan in the house was not used, and paths were watered twice-daily, to provide a high relative humidity. Fungicide sprays (rates as in Trial 1) were applied on 30 July when the level of mildew on all plants was approximately 10% leaf area affected. The leaf area affected by mildew was assessed at weekly intervals on the leaf immediately above fruit truss 12. All fruit was picked twice-weekly and total and marketable fruit yields were recorded.

Statistical analysis

The results of mildew assessments were examined by analysis of variance.

Development of a fungicide resistance test

Detached leaves

Detached tomato leaves, cv. Gardener's Delight or Pronto, were dipped in fungicide (Benlate, Nimrod or Rubigan), allowed to dry and inoculated with mildew spores of the test sample by using a cotton bud (Sadasisvan Nair & Ellingboe, 1962). Fungicides were tested at product label recommended rates and at three dilutions (1 in 2, 1 in 10 and 1 in 20). Inoculated leaves were incubated over water in an illuminated incubator at 23°C and observed for development of mildew pustules. A new cotton bud was used for each fungicide treatment. There were five replicate leaves per test and each leaf was inoculated at two sites. Controls consisted of leaves dipped in water and inoculated, and uninoculated leaves. Leaves were assessed after 6 & 12 days for the number of inoculation sites at which mildew had developed and for abundance of mildew development (0 to 5 scale according to proportion of inoculated area affected; 0 - nil; 1 - 1 to 20%; 2 - 21 to 40%; 3 - 41 - 60%; 4 - 61 - 80%; 5 - 81 - 100%). Factors which might affect development of mildew were investigated including variety, age and surface of test leaves.

Whole plants

Pot-grown plants of cv. Counter, approximately 30 cm in height, were sprayed with the test fungicide or water and allowed to dry. Fungicides were used at the same concentrations as on detached leaves. Small leaf discs (2 cm diameter) affected by mildew were placed in contact with treated leaves. There were three replicate inoculation sites per plant (a top leaf, a middle leaf and a bottom leaf), and three plants per fungicide treatment. Plants were then covered with a clear polythene bag, lightly tied around the base to maintain a high humidity, and placed in partial-shade on a glasshouse floor. The bags were removed after 48h and plants placed on a bench in an unheated glasshouse. Each set of plants inoculated with the test isolate, together with an un-inoculated control, were arranged in a randomised layout. Plants were examined for mildew at weekly intervals after inoculation and the number of sites at which mildew had developed was assessed.

Spore germination

Spores were collected by brushing mildew pustules with an artist's soft paintbrush. They were suspended in a bottle of sterile distilled water (SDW) by agitation of the paintbrush. Droplets of the spore suspension were then placed on a microscope slide previously coated with a thin layer of tap water agar (TWA), or agar plus fungicide at four concentrations (the same as used on detached leaves). Cover slips were applied and the slides were incubated in a humid chamber at 20°C. Slides were examined for the extent of spore germination after 12-18 hours. Spores were deemed to have germinated when they had produced a germ tube equal to or greater in length than the spore. Five replicate droplets were examined assessing 20 spores across a transect.

Source of mildew

Samples of leaves affected by mildew were obtained from commercial tomato nurseries and from ADAS Reading (92/1). Sample 92/1 had not been exposed to any fungicide for at least two years. For whole plant fungicide resistance tests, a sample (PV) obtained from a commercial crop in Essex was used; the crop had been sprayed once with Rubigan.

Production of fresh crops of young mildew spores

Mildew received from commercial nurseries was often grey-white in colour rather than the bright white colour of a young sporing pustule and it was difficult to obtain large quantities of spores for transfer to test leaves. Various methods were used to encourage spore production. In 1992, pustules were brushed with a dry brush to remove old spores and the leaves were then incubated in a warm, humid container. In 1993, samples of mildew from commercial crops were immediately inoculated on to untreated detached leaves of cv. Pronto, to sub-culture the fungus.

RESULTS

Phytotoxicity tests

None of the treatments had a detectable effect on growth or flower number 14 days after the second sprays were applied (Table 1). There was no evidence of leaf scorch or other damage. Sprays of Repulse at all three rates left a very visible deposit on leaves. Less noticeable deposit was left by sprays of Benlate, Elvaron and Thiovit and at the half rate it was barely visible (Table 1). Spray deposit left by Nimrod and Rubigan was barely visible even at x2 rate.

Just before the first sprays were applied, symptoms of powdery mildew arising from natural infection appeared and rapidly became widespread on plants in the experiment. Although not originally intended, opportunity was taken to assess the effects of the different fungicide treatments on incidence and severity of the disease.

Rubigan, Nimrod and Thiovit sprays checked powdery mildew even at x½ rate. Benlate, Repulse and Elvaron were also effective at x2 and x1 rates but control at the x½ rate was not quite so good. Spraying with water instead of fungicide also gave some control of the low level of mildew, but was less effective than any of the fungicide treatments (Table 2).

Table 1. Effect of fungicide sprays on flowering and deposit left on leaves

Treatment		Mean number of open flowers on first truss		Spray deposit (0 - 3)
		Pronto	Calypso	
Benlate	x2	5.2	3.4	1.3
	x1	5.0	2.8	1.0
	x½	4.8	4.2	0.3
Elvaron	x2	5.7	2.7	2.1
	x1	4.9	2.8	0.9
	x½	4.7	2.2	0.9
Nimrod	x2	4.9	2.7	0.1
	x1	5.3	3.1	0.1
	x½	5.8	3.3	0
Repulse	x2	4.5	3.1	3.0
	x1	5.1	3.8	2.8
	x½	5.4	4.3	1.9
Rubigan	x2	5.3	4.0	0
	x1	5.6	3.1	0.2
	x½	5.8	2.8	0.2
Thiovit	x2	5.8	3.4	2.0
	x1	4.9	3.2	1.9
	x½	5.0	2.8	1.0
Controls				
water		4.8	3.0	0
unsprayed		3.6	1.4	0

Assessed 2 weeks after the second spray.
Spray deposit: 0 - nil, 3-heavy deposit.

Table 2. Effect of fungicide sprays on powdery mildew

Treatment		% area of leaf 4 affected+ 30 Jan	6 Feb
Benlate	x2	0.05	0.05
	x1	0.02	0.02
	x½	0.35	0.35
Elvaron	x2	0.07	0.07
	x1	0.05	0.05
	x½	0.35	0.35
Nimrod	x2	0	0
	x1	0.02	0.02
	x½	0.07	0.07
Repulse	x2	0.07	0.07
	x1	0.05	0.05
	x½	0.20	0.20
Rubigan	x2	0.03	0.03
	x1	0.02	0.02
	x½	0.02	0.02
Thiovit	x2	0.03	0.03
	x1	0	0
	x½	0.05	0.07
Control	(water sprayed)	0.72	0.86
Control	(unsprayed)	9.00	12.83
Significance		0.05	0.05
SED (unsprayed control vs treatment)		1.17	1.81

Mildew appeared 6 January; sprays applied 8 and 23 January.
+ Counting up from the lowest true leaf.

Comparison of Fungicides - Trial 1

Treatment with fungicide significantly reduced mildew levels while treatment with water alone did not control the disease (Table 3). A single early spray of any fungicide, when approximately 20% of plants were affected at a very low level, gave good control which persisted for approximately 6 weeks (Fig. 1).

When the late sprays were applied mildew was present on at least two leaves on every plant and affected 2-3% leaf area per plant. Nevertheless, a single spray again resulted in good disease control, albeit for only about 3 weeks (Fig. 2). Spraying on both occasions gave very good control (Fig. 3) and at some assessments was superior to either of the single spray treatments (Table 5). No significant differences were detected when individual products and spray timings were compared (Table 6).

Comparison of Fungicides - Trial 2

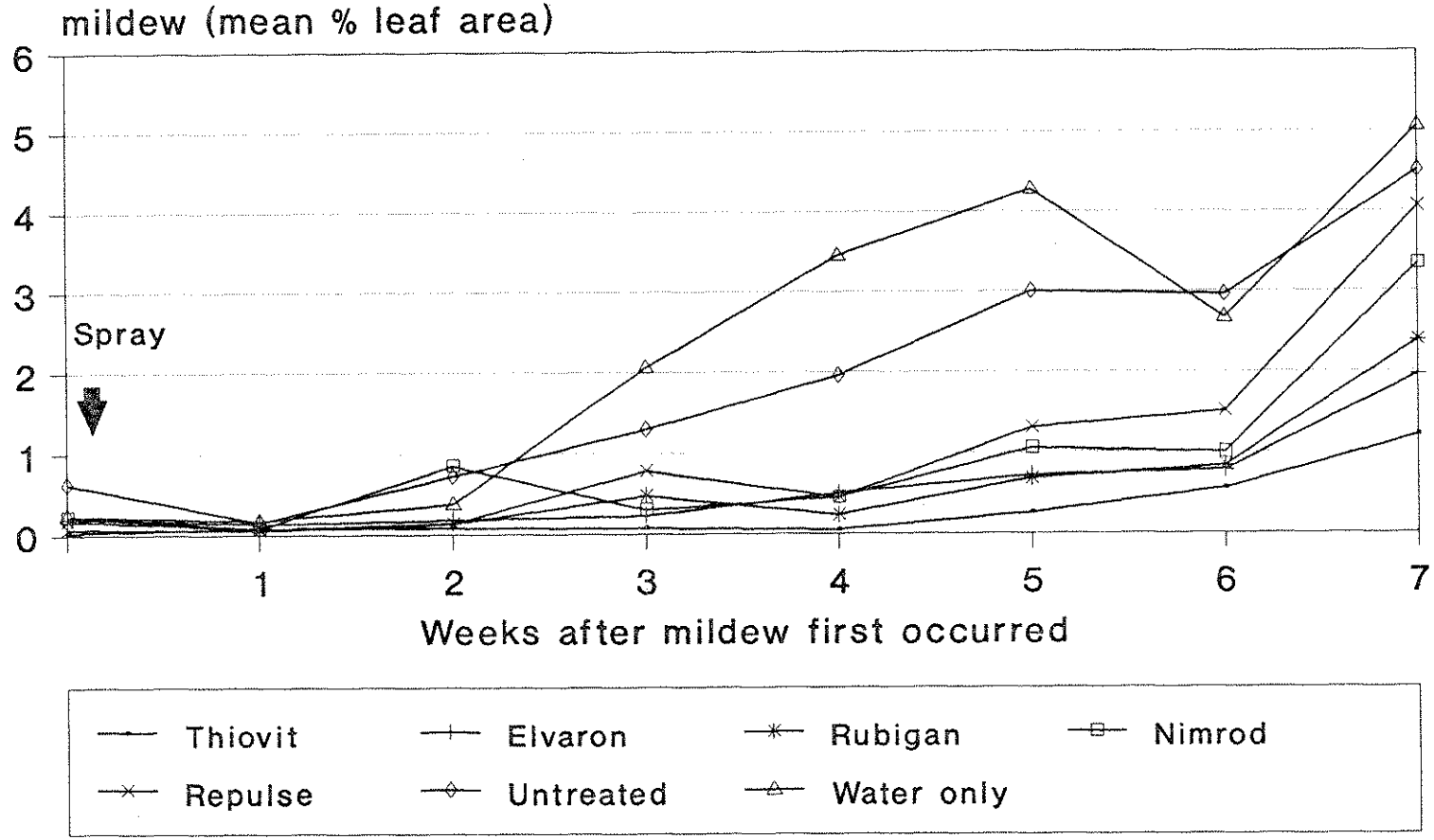
The objective of this trial was to compare the effectiveness of products applied when mildew was well established in a crop. When fungicide treatments were applied the mean leaf area affected by mildew was 9.8% (Table 7). The disease continued to increase rapidly in untreated plots and within a week affected over 60% leaf area. Fig 4 shows disease progress on the upper leaves. For more than three weeks after fungicide was applied mildew levels were significantly lower on all fungicide-treated plants than the untreated control. At the final assessment four weeks after fungicides were applied, the mildew level was significantly reduced by all treatments except Nimrod. The leaf area affected by mildew was least after Rubigan (19.6%), followed by Repulse (37.1%), Thiovit (37.9%), Elvaron (44.9%) and Nimrod (59.4%). In the untreated plots 86.4% of leaf area was affected.

None of the treatments resulted in a significant increase in fruit yield (Table 8).

These experiments clearly demonstrated that a range of products can give effective control of mildew. When the level of mildew at the time of treatment was relatively low, protectant fungicides (Thiovit, Repulse and Elvaron) appeared to be as effective as eradicants. When the initial disease level was relatively high, Rubigan appeared to be the best treatment and was significantly better than Nimrod.

Trial 1 - Comparison of fungicides

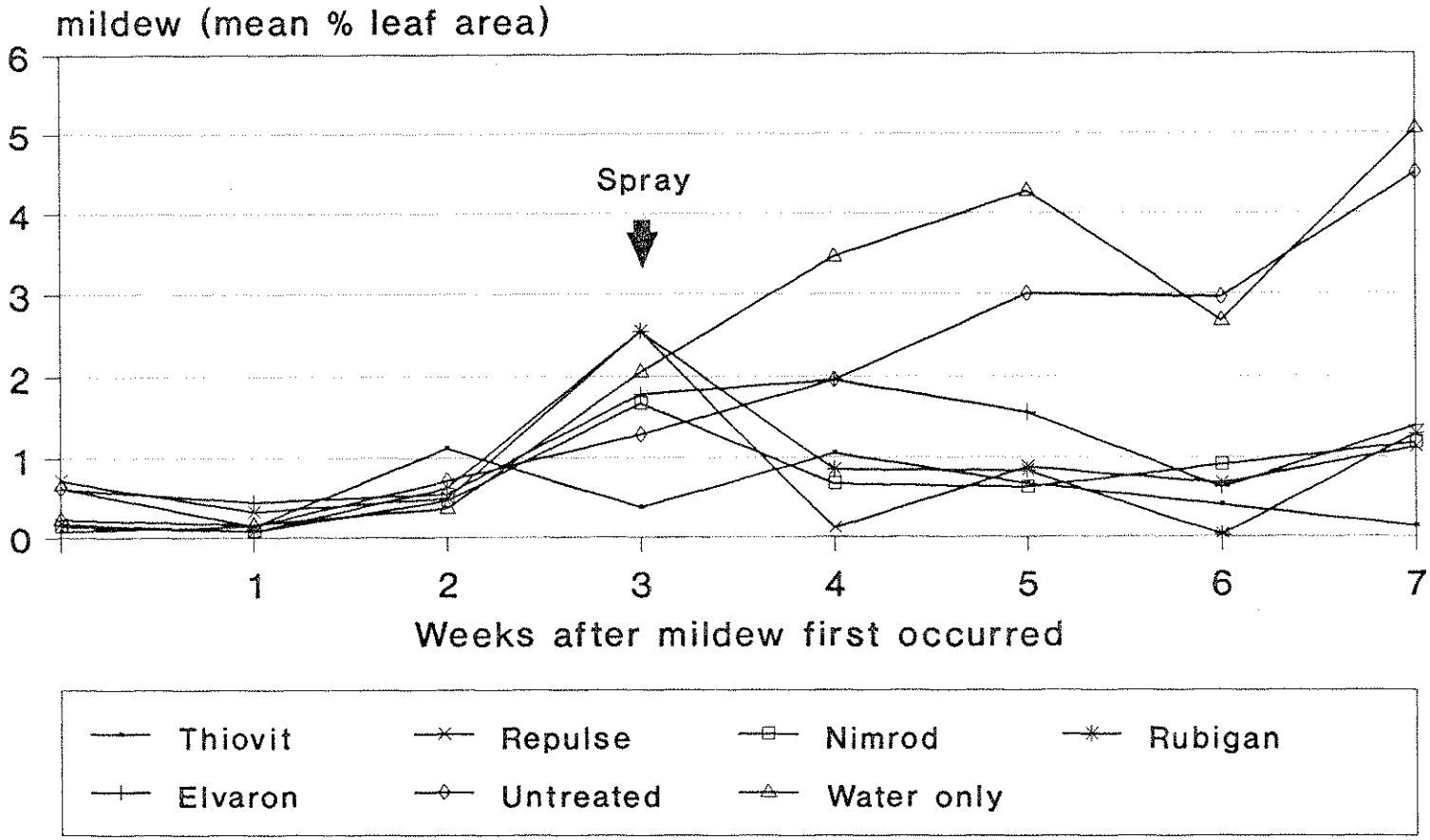
Early spray



TimSun2a

Trial 1 - Comparison of fungicides

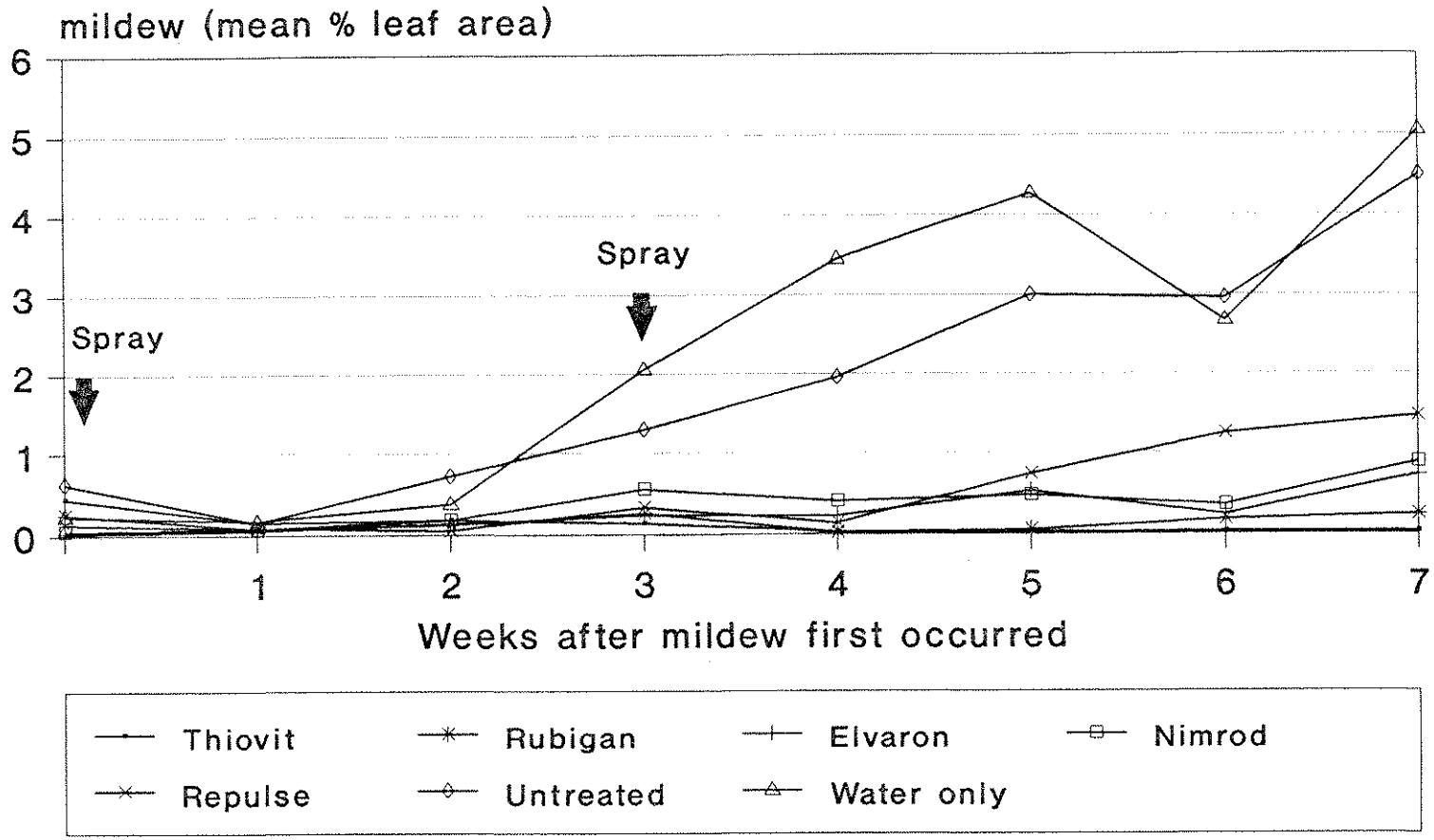
Late spray



TimSun2b

Trial 1 - Comparison of fungicides

Early + late spray



TimSun2c

Table 3. Comparison of untreated against fungicide treated - Trial 1

Treatment	No of Reps	Mildew (mean % of leaf area) Weeks after treatment 1	2	3	4	5	6	7	8
Fungicide	45	0.2	0.1	0.4	1.0	0.5	0.7	0.6	1.4
Untreated	3	0.6	0.2	0.7	1.3	2.0	3.0	3.0	4.5
Water	3	0.2	0.2	0.4	2.1	3.5	4.3	2.7	5.1
Significance		NS	NS	NS	NS	<.001	<.001	<.001	<.001
SED between control & water		0.28	0.13	0.39	0.83	0.65	0.88	0.53	0.95
control vs fungicide		0.21	0.09	0.29	0.61	0.47	0.64	0.39	0.69

Table 4. Comparison of fungicide products - Trial 1

Treatment	No of Reps	Mildew (mean % of leaf area) Weeks after treatment 1	2	3	4	5	6	7	8
Elvaron	9	0.3	0.2	0.3	0.8	0.9	0.9	0.6	1.4
Nimrod	9	0.1	0.1	0.5	0.8	0.5	0.7	0.8	1.8
Repulse	9	0.3	0.2	0.2	1.2	0.2	1.0	1.1	2.2
Rubigan	9	0.2	0.1	0.3	1.1	0.4	0.5	0.4	1.3
Thiovit	9	0.2	0.1	0.5	1.3	0.4	0.3	0.3	0.5
Untreated	3	0.6	0.2	0.7	1.3	2.0	3.0	3.0	4.5
Water	3	0.2	0.2	0.4	2.1	3.5	4.3	2.7	5.1
Significance		NS	NS	NS	NS	NS	NS	NS	NS
SED between control & water		0.28	0.13	0.39	0.83	0.65	0.88	0.53	0.95
control vs fungicide		0.23	0.10	0.32	0.68	0.53	0.72	0.43	0.77
between fungicides		0.16	0.07	0.23	0.48	0.37	0.51	0.31	0.55

Table 5. Comparison of fungicide timing - Trial 1

Treatment	No of Reps	Mildew (mean % of leaf area) Weeks after treatment 1	2	3	4	5	6	7	8
Early	15	0.1	0.1	0.3	0.4	0.3	0.8	0.9	2.6
Late	15	0.4	0.2	0.7	2.4	0.9	0.9	0.5	1.0
Early + Late	15	0.2	0.1	0.1	0.3	0.2	0.4	0.4	0.7
Untreated	3	0.6	0.2	0.7	1.3	2.0	3.0	3.0	4.5
Water	3	0.2	0.2	0.4	2.1	3.5	4.5	2.7	5.1
Significance		NS	NS	0.05	<0.001	0.05	NS	NS	<.001
SED between control & water		0.28	0.13	0.39	0.83	0.65	0.88	0.53	0.95
control vs fungicide		0.22	0.10	0.30	0.65	0.50	0.68	0.41	0.73
between fungicides		0.13	0.06	0.18	0.37	0.29	0.39	0.24	0.42

Table 6. Comparison of fungicide products and timing - Trial 1

Treatment	Mildew (mean % of leaf area)							
	Weeks after treatment							
	1	2	3	4	5	6	7	8
Early								
Elvaron	0.2	0.1	0.2	0.2	0.5	0.7	0.8	2.0
Nimrod	0.2	0.1	0.9	0.3	0.5	1.1	1.0	3.3
Repulse	0.1	0.1	0.1	0.8	0.5	1.3	1.5	4.1
Rubigan	0.2	0.1	0.1	0.5	0.2	0.7	0.8	2.4
Thiovit	T	0.1	0.1	0.1	0.1	0.3	0.6	1.2
Late								
Elvaron	0.6	0.5	0.6	1.8	2.0	1.6	0.6	1.4
Nimrod	0.2	0.1	0.5	1.7	0.7	0.6	0.9	1.2
Repulse	0.7	0.3	0.5	2.6	0.1	0.9	0.7	1.1
Rubigan	0.2	0.1	0.6	2.6	0.9	0.8	T	1.3
Thiovit	0.1	0.2	1.1	0.4	1.1	0.7	0.4	0.1
Early and late								
Elvaron	T	0.1	0.1	0.2	0.2	0.5	0.2	0.7
Nimrod	0.1	0.1	0.2	0.6	0.4	0.5	0.4	0.9
Repulse	0.1	0.1	0.1	0.3	0.1	0.7	0.2	0.2
Rubigan	0.3	0.1	0.1	0.3	T	0.1	0.2	0.2
Thiovit	0.5	0.2	0.2	0.1	T	T	T	T
Untreated	0.6	0.2	0.7	1.3	2.0	3.0	3.0	4.5
Water	0.2	0.2	0.4	2.1	3.5	4.3	2.7	5.1
Significance	NS	NS	NS	NS	NS	NS	NS	NS
SED	0.27	0.12	0.39	0.83	0.65	0.88	0.53	0.95

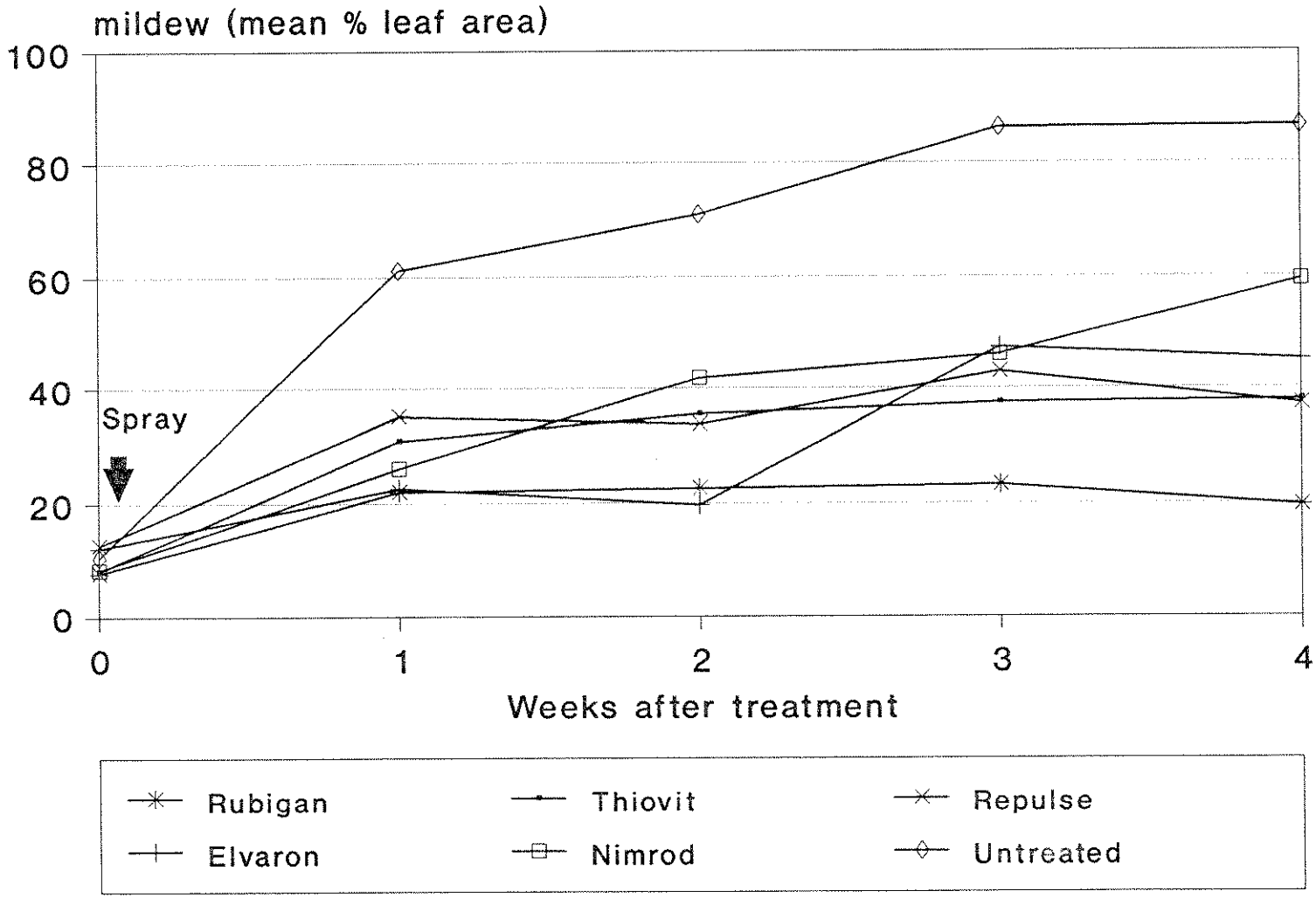
T - trace

Table 7. Comparison of fungicides - Trial 2

	Mean % leaf area affected				
	Weeks after treatment				
Treatment*	0	1	2	3	4
Untreated	10.4	61.2	71.1	86.1	86.4
Elvaron	12.1	22.5	19.6	47.2	44.9
Nimrod	8.2	26.0	41.8	45.8	59.4
Repulse	12.6	35.1	33.6	42.9	37.1
Rubigan	7.7	21.8	22.5	23.1	19.6
Thiovit	8.0	30.8	35.5	37.4	37.9
Significance	-	<0.05	<0.01	<0.01	<0.01
SED	-	11.91	11.39	11.98	15.17

Fungicides applied 30 July

Trial 2 - Comparison of fungicides



TimSun6

Table 8. Fruit yield 30 July - 27 August (Trial 2)

	Fruit yield (g/plant)					
	Weeks after treatment					
	1	2	3	4	4 (Green)	Total
Untreated	902	221	95	154	566	1938
Elvaron	337	318	285	184	849	1973
Nimrod	423	140	160	303	734	1760
Repulse	403	200	271	126	1032	2032
Rubigan	565	265	67	125	883	1905
Thiovit	461	241	249	175	909	2036
Significance	NS	NS	NS	NS	NS	NS
SED	234.1	111.8	96.9	122.9	355.3	791.2

Fungicide resistance testing

Detached leaf tests - 1992

Mildew developed consistently when spores were transferred from young freely-sporing pustules but not when older discoloured pustules were used. Results for sample 92/1, tested in May 1992, on leaves dipped in Benlate, Nimrod or Rubigan at full, ½, 1/10th and 1/20th recommended spray rates, are shown in Table 9.

Table 9. Sensitivity of sample 92/1 to Benlate, Nimrod and Rubigan

Treatment and rate	Number of inoculation sites with mildew (out of 10)		Mean disease score	
	6 days	12 days	6 days	12 days
Water	10	10	4.6	5.0
Benlate (x 1)	0	0	0	0
(x ½)	0	0	0	0
(x 1/10)	0	0	0	0
(x 1/20)	0	0	0	0
Nimrod (x1)	1	1	0.2	0.3
(x½)	2	3	0.3	0.6
(x1/10)	4	10	0.4	1.8
(x 1/20)	5	10	0.6	2.4
Rubigan (x1)	1	1	0.1	0.1
(x½)	0	1	0	0.2
(x1/10)	0	1	0	0.1
(x 1/20)	7	9	1.9	2.5
Uninoculated control	0	0	0	0

Six days after inoculation this isolate was found to be sensitive to all three fungicides applied at normal rate and notably sensitive to Benlate (Table 9). It was less sensitive to Nimrod and Rubigan at reduced rates. Ten samples of mildew-affected leaves received from commercial nurseries during June were tested using the method described above but none established, even on leaves dipped in water. Further tests were therefore carried out to try and determine the cause of mildew failing to establish.

The variety, age and surface of test leaves and methods of packing and posting mildew affected leaves (isolate 92/1) were investigated as possible reasons. Mildew established more consistently on leaves of cv. Gardener's Delight (39/40 inoculation sites) than cv. Pronto (28/40 sites). The success of establishment was not affected by leaf surface (22/40 on upper; 23/40 on lower) or method of packing and posting (22/40 following posting in a polythene bag; 26/40 following posting in a dry paper towel). There was slightly better establishment on young leaves of cv Pronto (9/40) than on old leaves (3/40) of the same variety. Three further isolates from commercial nurseries were tested on young leaves of cv. Gardener's Delight and failed to establish.

Detached leaf tests - 1993

Samples received between May and August 1993 from 13 commercial nurseries were transferred to untreated detached leaves and incubated. Three samples established well and were subsequently tested on detached leaves treated with Rubigan. Results for sample 93/LV are shown in Table 10. The remaining two samples failed to establish.

Sample 93/LV was relatively insensitive to Rubigan and developed on most leaves treated at one-tenth the usual spray-rate.

Table 10. Sensitivity of sample 93/LV to Rubigan

Treatment and rate	Number of inoculation sites with mildew (out of 10)		Mean disease score	
	6 days	12 days	6 days	12 days
Water	10	10	4.7	5.0
Rubigan (x1)	0	0	0	0
(x½)	0	0	0	0
(x1/10)	6	8	0.8	0.8
(x 1/20)	10	10	3.7	3.7
Uninoculated control	0	0	0	0

Whole plant tests

Four weeks after inoculation all but one of the water-treated leaves was affected by mildew (Table 11). At this time both Rubigan and Nimrod were giving good control of the disease at $^1/_{10}$th but not at $^1/_{20}$ normal spray rates. Five weeks after inoculation Rubigan gave control at its full recommended rate but colonies developed on leaves treated with Nimrod at its full recommended rate.

Table 11. Comparison of Nimrod and Rubigan on powdery mildew development

Treatment	Rate	Number of sites (of 9) infected with mildew		
		Weeks after inoculation		
		3	4	5
Nimrod	Full rate	0	0	3
	1/2	0	0	2
	1/10	0	0	3
	1/20	0	2	6
Rubigan	Full rate	0	0	0
	1/2	0	0	2
	1/10	0	0	4
	1/20	0	4	5
Water control		6	8	8
Uninoculated		0	0	0

Inoculated 13 August 1992

Production of young pustules

Incubation of leaves in a damp chamber was unsuccessful as leaves were rapidly rotted by grey mould (*Botrytis cinerea*) or bacterial soft rot. Transfer of spores from old pustules to untreated detached leaves was generally unsuccessful in producing new pustules.

Spore germination

Spore germination declined as fungicide concentration increased and none of the isolates showed a high germination rate on agar amended with full-rate fungicide. The germination of samples received from Rubigan treated crops was similar to that of samples from untreated crops (Table 12).

Table 12. Percentage germination of different mildew samples placed on agar amended with Rubigan and Nimrod

Sample	Control	Rubigan rate *				Nimrod rate*			
	(TWA)	1/20	1/10	1/2	1	1/20	1/10	1/2	1
Crops not sprayed with Rubigan									
1	28	-	12	4	4	-	-	-	-
2	39	11	6	5	2	-	-	-	-
3	32	14	7	4	1	17	12	5	2
4	34	-	10	6	2	-	-	-	-
5	28	16	8	6	2	-	-	-	-
6	26	14	9	6	5	-	-	-	-
7	50	26	18	8	6	-	-	-	-
Mean	34	16	10	6	3	-	-	-	-
Crops sprayed with Rubigan									
1	52	32	20	12	8	-	-	-	-
2	24	14	12	4	0	-	-	-	-
3	57	22	17	5	4	15	11	6	5
Mean	46	23	16	7	4	-	-	-	-

* Full rates were: Rubigan: 9ml/100l; Nimrod 200ml/100l

DISCUSSION

The phytotoxicity tests indicated that young plants (7-true leaf stage) in low natural light can be safely treated with Benlate, Elvaron, Nimrod, Repluse, Rubigan or Thiovit plus Agral at label or SOLA specified rates. On older plants, there have been reports that treatment with Rubigan can result in a brown marking on green fruit. This symptom was not observed following treatment on 16 May, or on this date and again 17 days later.

The results of fungicide efficacy tests confirm and extend those made in the previous HDC Project (PC26) and by Fletcher *et al.* (1988). It was particularly striking that the protectant fungicides Elvaron, Repulse and Thiovit plus Agral appeared as effective as the eradicant fungicide Rubigan when applied at very low or low levels of mildew. Project PC26 found no significant difference between products applied soon after disease occurred in a crop, although treatments then consisted of spray application every 14 days. Fletcher *et al.* (1988) reported that the disease was well controlled by a range of fungicides including Benlate, Bavistin FL, Bravo 500, Nimrod and Rubigan.

Nimrod appeared to give a shorter persistence of control than Rubigan in both the second fungicide experiment and in the resistance tests. This has not been noted in previous work.

When fungicides were applied five days after the initial appearance of mildew a single spray of each product gave good disease control for approximately six weeks. When fungicides were applied 22 days after appearance of mildew, treatment took longer to become effective and the mildew was controlled for shorter lengths of time. Not surprisingly, spraying five and 22 days after the initial appearance of mildew gave very good and prolonged control of the disease.

When fungicides were applied to plants severely affected by mildew (Trial 2) all products gave moderately good disease control which persisted for at least four weeks. Four weeks after treatment the control achieved with Rubigan was significantly better than that achieved with Nimrod.

Yarwood (1939) reported control of some powdery mildews with a water spray. Sivapalan (1993) found that conidia of different *Erysiphe* spp. exhibited substantial variation in their response to water. Some species showed a similar germination in water to that on a leaf surface and others showed poor germination; tomato powdery mildew was not tested. In our experiments, spraying twice with water gave moderately good control of a low level of mildew on very young plants. However, there was no evidence that spraying older plants twice-weekly with water controlled tomato mildew. More frequent spraying may have a different effect.

Development of a rapid, accurate and reproducible method for evaluating the response of tomato powdery mildew to fungicides is important because it would help to ensure appropriate treatment to be selected. A detached leaf or leaf disc technique has been used

successfully for investigating *Sphaerotheca fuliginea* on cucumber and melon (Bent et al., 1971; Schepers, 1983). The detached leaf technique reported here for tomato mildew was relatively rapid (2 weeks) but was poorly reproducible. It is possible that a water film on leaves (Sivapalan, 1993) or a high light intensity (Cohen, 1993) may have affected establishment of mildew in these tests. Further work is required to define more precisely the conditions under which mildew spores will germinate and develop to produce sporing pustules on detached leaves.

The mean germination rate of mildew samples from fenarimol treated crops was similar to that of samples from untreated crops. The results of germination tests to measure fungicide sensitivity should be interpreted with caution as they indicate the effect of a fungicide at only one or two stages in the development of a pathogen. Nevertheless, the results for tomato powdery mildew do indicate that there is a wide range of sensitivities to fenarimimol within population.

When resistance to SBI fungicides occurs it generally develops in small steps making it difficult to determine when poor control is due to fungicide resistance rather than to other factors (Koller & Scheinpflug, 1987) . Reduced disease control of *S. fuliginea* was associated with an increase in the ED_{95} from 0.2 to 17 µg/ml (Huggenberger *et al.*, 1984). In the tests described here a range of sensitivities to fenarimol was found indicating that insensitive strains may exist in greenhouse populations. With frequent use of fenarimol insensitive strains may become predominant. However, further samples of mildew, collected from crops where different degrees of control have been observed, need to be tested in order to confirm that resistance has occurred and to improve interpretation of tests on detached leaves. An examination of the range of sensitivities in populations using single pustule samples is also desirable. The sensitivity of tomato mildew to benomyl and bupirimate was similar to that reported by Fletcher *et al.*, (1988), who observed no mildew on tomato plants treated with benomyl at concentrations as low as 100 µg/ml, and a small amount of mildew at all concentrations of bupirimate up to 370 µg/ml.

The speed of mildew development was slower on young plants (3 - 4 weeks) than on detached leaves (6 days), probably because the former tests were done at ambient temperature. It is suggested that the critical time for assessment in sensitivity tests is as soon as mildew is present at most inoculation sites on untreated leaves or plants.

CONCLUSIONS

1. Benlate, Elvaron, Nimrod, Repulse, Rubigan and Thiovit plus Agral applied in January to young tomato plants did not affect plant growth or flowering and did not cause a leaf scorch.

2. Repulse left a very visible spray deposit on plants. Rubigan and Nimrod left no visible spray deposit; other fungicides were intermediate.

3. A single spray of Elvaron, Nimrod, Repulse, Rubigan or Thiovit plus Agral applied five days after mildew occurred in a crop all gave good disease control for approximately six weeks. There was no significant difference between treatments.

4. The same fungicides applied when all plants showed 1-2% leaf area affected by mildew again gave good disease control and reduced the disease to low levels. There were no significant differences between treatment. Application of fungicides at both timings reduced the disease to very low levels.

5. A single spray of the same fungicides applied when plants were severely affected by mildew (10% leaf area affected) reduced the rate of disease increase, compared to that on untreated plants. Four weeks after treatment the leaf area affected by mildew was significantly less with Rubigan (19.6%) than Nimrod (59.4%).

6. Spraying young plants with water gave good control of a very low level of mildew but spraying older plants twice-weekly with water was ineffective.

7. Freely-sporing samples can be tested for fungicide resistance on detached tomato leaves or young tomato plants treated with fungicide at a range of concentrations. The tests take approximately 2 and 4 weeks respectively from inoculation to final assessment .

8. A range of senstivities to Rubigan was found.

9. Samples of mildew received from commercial crops for resistance-testing were not freely-sporing and most failed to establish on test leaves. Attempts to improve establishment by altering variety, age and surface of test leaves, methods of packing and posting affected leaves from commercial crops and by producing fresh mildew pustules before testing were not successful. Further work is required to define more precisely the conditions under which mildew spores will germinate and develop to produce sporing pustules on detached leaves.

10. Incubation of leaves with poorly sporing pustules in a humid chamber to try and increase spore number resulted in leaf rot from botrytis before fresh crops of spores could be produced.

11. The germination rate of mildew spores on agar containing Nimrod or Rubigan declined as the concentration of fungicide increased. A small proportion of spores (<5%) germinated on agar containing full rate Nimrod or Rubigan.

12. The germination of three samples obtained from crops treated with Rubigan was similar to that of seven samples obtained from crops not treated with Rubigan.

13. In both the fungicide resistance tests and the spray trials the persistence of disease control with Rubigan appeared to be greater than that with Nimrod at the rates tested.

REFERENCES

Bent, K.J., Cole, A.M., Turner, J.A.W. & Woolner, M. (1971). Resistance of cucumber powdery mildew to dimethirimol. *Proceedings of the 6th British Insectivide and Fungicide Conference*, 274 - 282.

Cohen, R. (1993). A leaf disc assay for detection of resistance of melons to *Sphaerotheca fuliginea* Race 1. *Plant Disease* **77**, 513 - 517.

Fletcher, J.T., Smewin B.J. & Cook R.T A. (1988). Tomato powdery mildew. *Plant Pathology* **37**, 594-598.

Huggenberger, F., Collins, M.A. & Skylakakis, G. (1984). Decreased sensitivity of *Sphaerotheca fuliginea* to fenarimol and other ergosterol-biosynthesis inhibitors. *Crop Protection* **3**, 137 - 149.

Koller, W. & Scheinpflug, H. (1987). Fungal resistance to sterol biosynthesis inhibitors - a new challenge. *Plant Disease* **71**, 1066 - 1074.

Sadasivan Nair K.R. & Ellingboe A.H. (1962). A method of controlled inoculation with conidiospores of *Erysiphe graminis* var. *tritici*. *Phytopathology* **52**, 714.

Schepers H.T.A.M. (1983). Decreased sensitivity of *Sphaerotheca fuliginea* to fungicides which inhibit ergosterol biosynthesis. *Netherlands Journal of Plant Pathology* **89** 185 - 197.

Schepers H.T.A.M. (1985). Fitness of isolates of *Sphaerotheca fuliginea* resistant or sensitive to fungicides which inhibit ergosterol biosynthesis. *Netherlands Journal of Plant Pathology* **91**, 65 - 76.

Schroeder, W.T. & Provvidenti, R. (1969). Resistance to benomyl in powdery mildew of cucurbits. *Plant Disease Reporter* **53**, 271 - 275.

Sivapalan, A. (1993). Effects of water on germination of powdery mildew conidia. *Mycological Research* **97**, 71 - 76.

Yarwood, C.E. (1939). Control of mildew with a water spray. *Phytopathology* **29**, 288 - 290.

ACKNOWLEDGEMENTS

Grateful thanks are given to the tomato growers who supplied samples of mildew-affected leaves during 1992 and 1993.

Contract between ADAS (hereinafter called the "Contractor") and the Horticultural Development Council (hereinafter called the "Council") for a research/development project.

PROPOSAL

1. TITLE OF PROJECT **Contract No: PC/26a**

FUNGICIDE SENSITIVITY TESTING AND COMPARISON OF FUNGICIDES FOR CONTROL OF TOMATO POWDERY MILDEW

2. BACKGROUND AND COMMERCIAL OBJECTIVES

Powdery mildew on glasshouse grown tomatoes is now widespread and many growers must resort to fungicide spraying to prevent it causing significant yield loss and reduced fruit quality. Experience is showing that outbreaks of the disease can occur at any time from propagation on and may occur more than once on the same nursery during the season. Consequently, some growers are needing to apply several sprays for control during this period.

Unfortunately there are no reliable alternatives to spraying for control of mildew. Limited evidence indicates that fenarimol as Rubigan and bupirimate as Nimrod are the most effective chemicals for controlling the disease although others such as dichlofluanid (as Elvaron), chlorothalonil (as eg Bombardier, Bravo 500 and Repulse) and benomyl (as Benlate) which all are registered for use on tomatoes against botrytis, can also give useful protection. How well these fungicides would perform under prolonged or heavy disease pressure is uncertain.

On a high value crop such as glasshouse grown tomatoes, growers naturally want to use the most effective products permitted. However, both fenarimol and bupirimate have a very specific (single site) mode of action which experience with other powdery mildews has shown is likely to result in resistance problems if either of these fungicides is used intensively. The same is also probably true with benomyl which we suspect is less likely to cause damage to young plants treated during the winter.

When outbreaks of powdery mildew occur during the winter there are fears that spraying with either Nimrod or Rubigan may cause damage. Both products are permitted as specific off label approvals and such use is at growers own risk.

3. POTENTIAL FINANCIAL BENEFIT TO THE INDUSTRY

Grower experience in 1988 & 1989 indicated that 2 or 3 well-timed sprays of a fungicide were often sufficient to eliminate powdery mildew. On a few nurseries however, it appeared that a regular spray programme was required to maintain mildew at a low level. In the last 2 years the disease appears to have become more common; it is now present on some nurseries throughout the season, and

growers and advisers are reporting that some fungicides are not providing adequate control.

As yet there has been no attempt made to measure yield reduction attributable to powdery mildew but a reasonable and probably conservative assumption can be made that the disease will soon be so widespread that it is likely to appear at some stage during the season on many if not most, commercial tomato nurseries. The disease can rapidly become severe enough to check growth if left untreated, and a 1-2% yield reduction, valued at £1 million nationally, is probably a conservative one if affected crops remained untreated.

A good soundly based and durable fungicide strategy will be very cost effective, preventing most of this loss, and at the same time encouraging rational and responsible use of fungicides.

The experiments listed, if successful, will provide the information needed to achieve these aims.

4. SCIENTIFIC/TECHNICAL TARGET OF THE WORK

A. To devise a practical means for testing tomato powdery mildew isolates for resistance to fenarimol, bupirimate and benomyl.

B. To use the devised technique to monitor the sensitivity of powdery mildew isolates obtained from commercial crops where these fungicides have been used to differing extents.

C. To examine the relative effectiveness of a range of fungicides (eg Benlate, Elvaron, Repulse, Rubigan, Nimrod, Thiovit) by testing them under conditions of heavy and prolonged disease pressure (conditions which have not prevailed in previous testing).

D. To monitor the effects of the fungicide when used in the above trial for adverse effects on biological control of whitefly and spider mite.

E. To check the sensitivity of young tomato plants of a range of cultivars to fungicide treatment during the winter.

5. CLOSELY RELATED WORK

The proposed study follows on from the 1990 HDC project on tomato powdery mildew (PC26) and complements the current MAFF funded work at ADAS Reading investigating the effects of relative humidity and temperature on the various stages of the powdery mildew disease cycle, and host range of tomato powdery mildew.

6. DESCRIPTION OF THE WORK

* Devise a reliable "laboratory technique" for checking sensitivity of tomato powdery mildew isolates to fungicides (Winter 1991/92).

* Use the technique to check isolates collected from commercial tomato crops at various times throughout the season (early 1992 - Autumn 1992, and again in 1992-1993 season).

* Undertake glasshouse experiments to check effect of permitted fungicides on young tomato plants propagated during winter (Winter 1991/92).

* Undertake glasshouse experiment to evaluate fungicides at different rates and frequencies on control of powdery mildew under heavy and prolonged infection pressure (Spring - Summer 1992); and to monitor the effect of these programmes on biological pest control.

7. COMMENCEMENT DATE AND DURATION

January 1992- November 1993
Final report by March 1994

8. STAFF RESPONSIBILITIES

Project Leader:

Dr T M O'Neill
ADAS Plant Pathology
Cambridge RO
Tel: 0223-455852
Fax: 0223-455911

D Ann
ADAS Plant Pathology
Reading RO
Tel: 0734-392289

9. LOCATION

ADAS laboratories and glasshouses at Reading and Cambridge

Contract No: PC/26a

TERMS AND CONDITIONS

The Council's standard terms and conditions of contract shall apply.

Signed for the Contractor(s)

Signature.....

Position R + D MANAGER

Date 17/2/92

Signed for the Contractor(s)

Signature.....

Position.....

Date.....

Signed for the Council

Signature.....

Position CHIEF EXECUTIVE

Date 11.2.92