Project title:	Outdoor lettuce: evaluation of novel fungicides for downy mildew control
Project number:	FV 357
Project leader:	Dr Peter Gladders, ADAS
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Key staff:	Dr K. Green, ADAS Ms A. Huckle, ADAS Ms L. Kirkpatrick, ADAS
Location of project:	ADAS Boxworth, Cambs
Project coordinator:	Mr David Norman, Fresh Produce Consultancy, March, Cambs.
Date project commenced:	1 April 2009
Date project completed (or expected completion date):	31 March 2010
Key words:	Lettuce, downy mildew, *Bremia lactucae*, fungicides, efficacy, timing

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

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GROWER SUMMARY

Headline

Invader (dimethomorph + mancozeb), Previcur Energy (fosetyl-aluminium + propamocarb hydrochloride), Revus (mandipropamid), Signum (boscalid + pyraclostrobin), Valbon (benthialvalicarb-isopropyl + mancozeb) and a coded product (HDC F3) were the six products that gave good control of lettuce downy mildew (*Bremia lactucae*) in an artificially inoculated pot experiment, out of the eleven fungicides tested.

Background and expected deliverables

Downy mildew caused by the oomycete pathogen *Bremia lactucae* is the most important disease problem in field-grown lettuce. Some varieties are resistant but major gene resistance can be overcome quite rapidly. Fungicides are thus required to support genetic resistance to maintain control of this disease. No screening of novel products for lettuce downy mildew control has occurred in the UK for almost 10 years despite significant developments in potato blight fungicides and related products during this same period. There is commercial support to extend the use of some of the products on lettuce and other vegetables. The project aimed to provide new information on fungicide efficacy to help growers improve on disease management to reduce wastage. The work complemented studies on other lettuce diseases in HDC funded projects that aim to provide growers with up-to-date information on the control of major lettuce pathogens.

Spray timing is likely to be critical for downy mildew control as its latent period is only a few days and frequent applications may be required to protect new growth. Programmes at 10 versus 14 days and at different timings in relation to infection events (protectant and curative), would identify weaknesses in product efficacy and persistence. Testing and ranking of fungicides enables growers to select the best products for the range of diseases they encounter. The current choice of fungicides is limited so growers need a selection of products to combine in programmes to reduce the risks of selecting fungicide resistant strains of the disease. Results from this project will also be useful for product selection on other crops.

The overall aim of the project was to determine the efficacy of new and standard fungicides for control of downy mildew in outdoor lettuce. The specific objectives were:

1. To confirm methods that ensure consistent development of lettuce downy mildew in screening experiments, using artificial inoculation with *Bremia lactucae* and; to produce inoculum sufficient for use in objective 2;
2. To determine the crop safety and activity of novel and standard fungicides against lettuce

downy mildew;

3. To define the dose response activity of the most promising products and;

4. To quantify the persistence of products, to guide timing of treatments.

Summary of the project and main conclusions

Three experiments were done to determine the efficacy of a range of existing and novel fungicides against lettuce downy mildew (*B. lactucae*), in terms of timing, dose and persistence. The three experiments were run in an unheated polythene tunnel at ADAS Boxworth, Cambs from June to October 2009, and managed to provide conditions conducive for downy mildew development. The cos lettuce variety Frisco was used throughout (Figure 1), as this was observed to be susceptible to lettuce downy mildew in previous experiments. In each experiment, lettuce blocks were transplanted into F2+S compost in 13 cm diameter pots (3 pots of 4 plants per plot). Inoculum of *B. lactucae* was applied to plants as a spore suspension prepared from a combination of fresh or frozen leaves with sporulating downy mildew. Following inoculation, plants were covered with a polythene 'tent' for 48 hours to maintain leaf wetness and to increase relative humidity. Fungicides were applied to plants at different timings in relation to artificial inoculation. Disease incidence (proportion of plants affected), disease severity (percentage plant area affected) and phytotoxicity were assessed at intervals after inoculation and spray applications.

Fungicides used in Experiment 1 were selected based on current availability of products on lettuce, and other potential fungicide products for downy mildew control following discussions with the grower coordinator and agro-chemical industry contacts,.

Figure 1. Sporulation of downy mildew on lettuce var. Frisco (left) and set-up of Experiment 3 in a polytunnel (right)

Fungicide efficacy and timing

In Experiment 1, 11 fungicides were applied either 5 days before (26 June), 2 days before (29 June), immediately prior to artificial inoculation (1 July), or 2 days after inoculation on 3 July (Table 1).

Table 1. Fungicide products included in Experiment 1

	Product	Active ingredient	Product rate / ha
1	Untreated control	-	-
2	Amistar	Azoxystrobin	1.0 L
3	Aliette 80 WG	Fosetyl-aluminium	3.0 kg
4	Fubol Gold	Mancozeb + metalaxyl-M	1.9 kg
5	Invader	Dimethomorph + mancozeb	2.0 kg
6	Previcur Energy	Fosetyl-aluminium + propamocarb hydrochloride	2.5 L
7	Revus	Mandipropamid	0.6 L
8	Signum	Boscalid + pyraclostrobin	1.5 kg
9	HDC F 1	Not disclosed	2.5 L
10	HDC F 2	Not disclosed	0.8 L
11	HDC F 3	Not disclosed	2.5 kg
12	Valbon	Benthialvalicarb-isopropyl/mancozeb	1.6 kg

Notes:

Amistar	SOLA 1465/01 for outdoor lettuce
Aliette 80 WG	SOLA 3522/06 for outdoor lettuce
Fubol Gold	SOLA 2142/03 for outdoor lettuce
Invader	SOLA 3044/06 for outdoor lettuce
Previcur Energy	SOLA 0513/04 for outdoor lettuce
Revus	Administrative Experimental Approval COP 2009/00865
Signum	On-label for outdoor lettuce
HDC F1	Administrative Experimental Approval COP 2009/00865
HDC F2	Administrative Experimental Approval COP 2009/00865
HDC F3	Administrative Experimental Approval COP 2009/00865
Valbon	Administrative Experimental Approval COP 2009/00865

At 1 week after inoculation, slight phytotoxicity was observed on the plants as scorch or yellow blotches but this was not specific to fungicide treatments. There was a lower incidence of phytotoxic symptoms on treatments that had received the final spray application (2 days after inoculation), due possibly to cooler conditions at the time of this fungicide application.

Symptoms of downy mildew were first observed 13 days after inoculation. Mean downy mildew incidence was 12% in the untreated control at 15 days after inoculation and had increased to 19% by 21 days after inoculation. There was a significant effect of timing at both assessments, irrespective of fungicide treatment, with lower downy mildew incidence resulting from fungicide treatment at -2, 0 and +2 day timings compared with treatment 5 days before inoculation (P=0.006). At 21 days after inoculation, all of the fungicides except Fubol Gold had reduced downy mildew incidence by at least half compared with the untreated control. Signum, Valbon and Previcur Energy were the best performing products, followed by Revus, Invader and HDC F3, all reducing disease incidence compared with

Fubol Gold (*P*<0.029) and the untreated control (Figure 2). There was no significant fungicide x timing interaction effect. Disease severity remained low throughout the experiment (mean of less than 0.5% in the untreated control) and there were no significant differences between fungicide treatments.

Fungicides to include in the 2nd experiment were selected largely on the basis of their effects on downy mildew incidence, since they could not be sufficiently differentiated using disease severity data. Previcur Energy, Revus, Signum and Valbon were selected as the most effective products. Invader and HDC F3 were similar to each other in efficacy, so Invader was selected for further experimentation since it is currently available for industry use. The poor performance of Fubol Gold (no different from the untreated control) in Experiment 1 was possibly due to use of a strain of *B. lactucae* with resistance to metalaxyl-M.

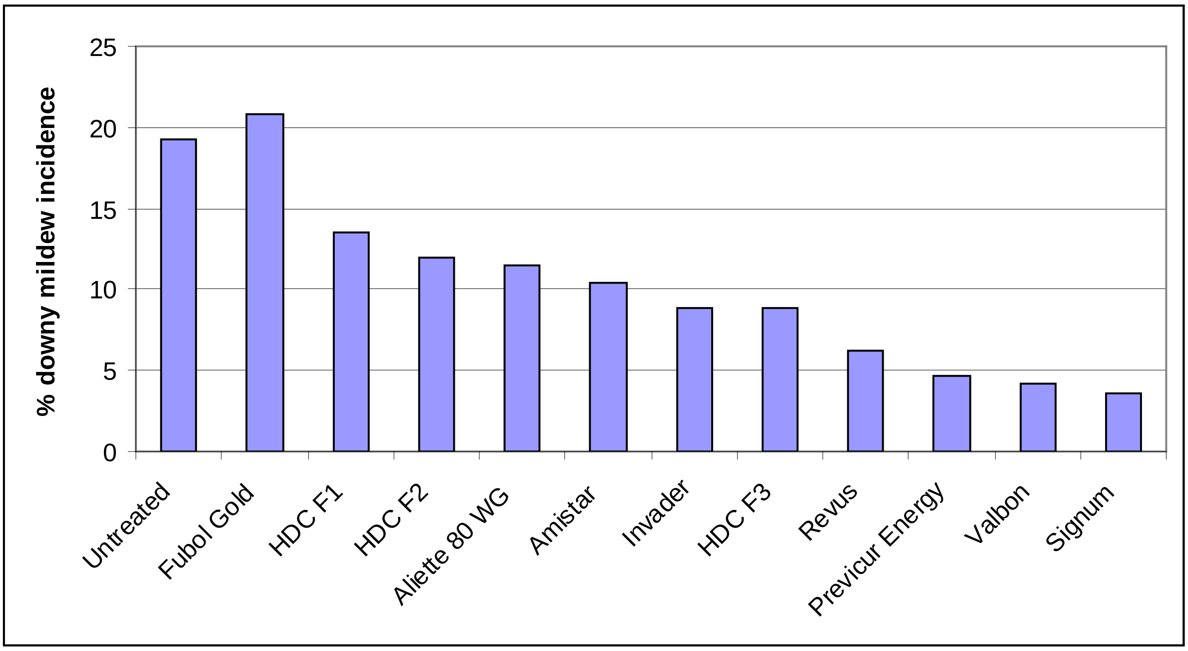


Figure 2. Effect of fungicides on incidence of lettuce downy mildew in Experiment 1 (averaged over four timings), 21 days after inoculation (LSD = 9.8)

Fungicide dose

Five products selected from Experiment 1 were evaluated to confirm efficacy and to identify the most effective products in relation to dose. The use of half dose treatments was done to help define the robustness of the treatments rather than to pursue low rates of application. Each fungicide treatment (Table 2) was applied at full rate or half rate at one of four different timings (-5, -2, 0 or +2 days in relation to inoculation).

No symptoms of phytotoxicity were observed in this experiment. Disease incidence increased from 11% in the untreated control at 7 days after inoculation to 84% at 16 days after inoculation. At 16 days after inoculation, there was a significant fungicide x timing interaction (P<0.001), when disease incidence was averaged across doses. This was due largely to a decrease in disease incidence when Invader and Valbon were applied 5 days before inoculation. Disease severity in the untreated control was higher than that recorded in Experiment 1. Fungicides significantly decreased downy mildew severity and there were significant differences between fungicides, but no effect of fungicide dose (Table 2).

There was a significant interaction effect of fungicide x dose on disease severity (P=0.046). Invader and Valbon were found to be more effective when applied at half dose, while Previcur Energy, Revus and Signum were similarly effective when used at full or half dose.

Table 2. Effect of fungicide doses on severity of downy mildew, 16 days after inoculation (Experiment 2)

	Fungicide treatments	Mean % disease severity		Fungicide means
		Full dose	Half dose	
1	Untreated control	-	-	3.1
2	Water only control	-	-	2.0
3	Invader	1.8	0.8	1.3
4	Previcur Energy	1.3	1.2	1.2
5	Revus	1.1	1.3	1.2
6	Signum	1.9	2.2	2.0
7	Valbon	1.9	1.0	1.5
	Dose means	1.6	1.3	
	LSDs (144 df)			
	Fungicides v. untreated	0.64		
	Fungicides only	0.52		
	Fungicides.dose	0.74		
	Dose	Ns		

Fungicide persistence

A third inoculated experiment was done to define the persistence of disease control in relation to timing of fungicide programmes. An interval of either 10 or 14 day between the two sprays was used for the most promising five products at full rate. Two infection events were included in this experiment. The five fungicide treatments were applied on the day of 1st inoculation (day 0) and either 10 days later (immediately before 2nd inoculation) or 14 days later.

At the 10 day spray timing, Revus was applied instead of Signum, and vice versa, in error; all other spray applications were correct. The data for these plots was included in statistical analyses and is shown in the results as Revus / Signum, or Signum / Revus programmes.

No phytotoxicity was observed in this experiment. Disease development was rapid with 71% downy mildew incidence in the untreated control by 10 days after 1st inoculation. Treatment differences were already visible at 14 days after the 1st inoculation (Figure 3). At 20 days, disease incidence was less than 50% for all of the fungicide treatments compared with 100% in the untreated control (Table 3). Up until and including 27 days after inoculation, all of the fungicides still significantly reduced disease incidence in comparison with the untreated and water controls. At 34 days, disease incidence was still significantly lower than the controls for the two Invader programmes and the Revus programme at 0 & 14 days. Irrespective of fungicide timing, the Previcur Energy treatments, and programmes commencing with Signum were less effective in reducing downy mildew incidence at 14 and 20 days after inoculation compared with Invader, Revus (and Signum), and Valbon. Invader was the most effective product over the duration of the experiment.

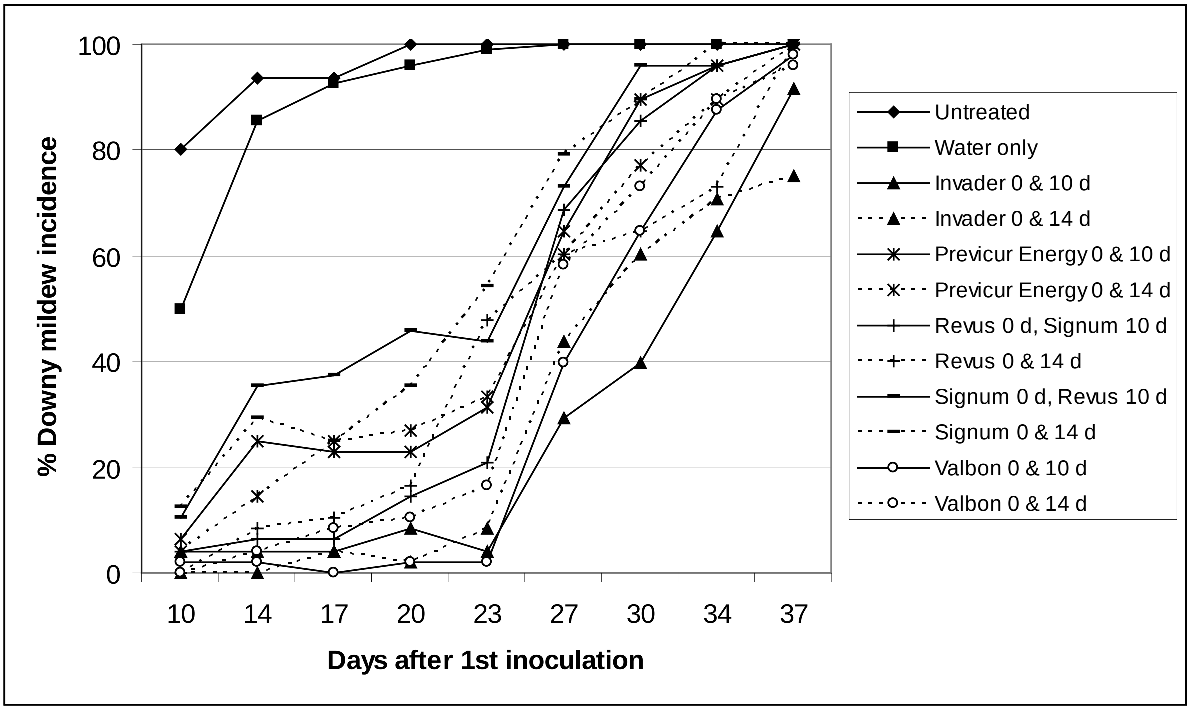
Disease severity increased to 18% of leaf area affected at 34 days after the 1st inoculation compared with 7% or less in the fungicide treatments (data for this is shown in the full report: table 11 & figure 3). The fungicide treatments significantly reduced disease severity in comparison with the controls at all of the assessment dates. Up until 27 days, disease severity remained low (1.6% or less), irrespective of fungicide product or spray intervals. At 34 days, disease severity was lower for the Invader programmes and Valbon programmes compared with Signum (0 & 14 days).

Invader was also the only product for which a spray interval of 10 days gave significantly better control than a 14 day interval; for the other products, downy mildew incidence did not vary significantly with spray timing interval. However, from looking at Figure 4, it is apparent that the rate of disease development on fungicide-treated plants was generally more rapid from day 20 after 1st inoculation. A similar trend was observed for disease severity data. This would suggest that in a field situation, a third spray at day 20 (ie 10 day spray intervals) might have checked disease development more than 14 day spray intervals, under a situation of high disease pressure.

Figure 3. Lettuce treated with Valbon (left) compared with untreated control plants, 14 days after 1st inoculation

Table 3. Effect of fungicide timing intervals on incidence of downy mildew (Experiment 3)

	Fungicides	Timing	% downy mildew incidence at intervals after inoculation			
			14 days	20 days	27 days	34 days
1	Untreated control	-	93.8	100.0	100.0	100.0
2	Water control	-	85.4	95.8	100.0	100.0
3	Invader x 2	0 & 10 days	4.2	8.3	29.2	64.6
4	Invader x 2	0 & 14 days	0.0	2.1	43.7	70.8
5	Previcur Energy x 2	0 & 10 days	25.0	22.9	64.6	95.8
6	Previcur Energy x 2	0 & 14 days	14.6	27.1	60.4	89.6
7	Revus/Signum	0 & 10 days	6.3	14.6	68.7	95.8
8	Revus x 2	0 & 14 days	8.3	16.7	60.4	72.9
9	Signum/Revus	0 & 10 days	35.4	45.8	72.9	95.8
10	Signum x 2	0 & 14 days	29.2	35.4	79.2	100.0
11	Valbon x 2	0 & 10 days	2.1	2.1	39.6	87.5
12	Valbon x 2	0 & 14 days	4.2	10.4	58.3	89.6
	LSDs (41 df):					
	Controls vs fungicides		11.90	12.72	18.44	18.31
	Fungicides		13.74	14.79	21.29	21.14



Note: 2nd inoculation at 10 days after 1st inoculation

Figure 4. Effect of fungicides on incidence of downy mildew at intervals after first inoculation (Experiment 3)

Financial benefits

Problems with downy mildew continue in all lettuce producing areas. A national loss in production of 10% is worth about £4 million/annum. Judicious use of effective fungicides can help to reduce this loss.

Action points for growers

- Of the fungicides currently approved for use on outdoor lettuce: Invader, Previcur Energy and Signum all have good activity against lettuce downy mildew, without causing phytotoxicity.
- The efficacy of Fubol Gold against lettuce downy mildew may be reduced if strains of *B. lactucae* resistant to metalaxyl-M are present.
- Valbon (approved for onion downy mildew) and Revus (approved for potato blight), were also effective against lettuce downy mildew in this project. Further work on residues may be warranted to determine whether Specific Off Label approvals for use of these fungicides on outdoor lettuce could be sought.

SCIENCE SECTION

Introduction

Downy mildew caused by the oomycete pathogen *Bremia lactucae* is the most important disease problem in field grown lettuce. Epidemics, and hence significant losses, occur regularly and can occur at any stage of the cropping programme. Complete crop losses do occur but average losses are more typically 10%.

Varietal resistance is available and can provide useful levels of control, although major gene resistance can be overcome quite rapidly. With the diversity of lettuce types now being grown and frequent changes in the pathogen population (FV 291: Update on *Bremia* resistance information for UK Growers, 2007), it is difficult to manage downy mildew with genetic resistance alone. Fungicides are therefore required to support genetic resistance to maintain control.

A diversity of fungicides is required to maintain control of lettuce downy mildew as strategies based on mixtures or alternation of products with different modes of action are required to reduce the risks of selecting of fungicide resistant strains. Insensitivity to metalaxyl-M by isolates of *B. lactucae* has been confirmed in several countries including the UK, while in California, isolates of *B. lactucae* were found to be resistant to fosetyl-aluminium.

Fungicide evaluation against lettuce downy mildew has been carried out at intervals on either protected or field crops (e.g. HDC Projects FV 95 (1991-95), PC 20 (1991), PC 20a (1998), FV 207 (1999), but there has been no screening of novel products for almost 10 years. In recent years, there have been significant new developments in potato blight fungicides and independent data on their effectiveness is available from the Potato Council and a network of European researchers.

There is commercial support to extend the use of some of these blight products into lettuce and other vegetables. New active ingredients and existing products that have both scope for downy mildew control and that could be progressed on field vegetables, have been identified for evaluation in this project in consultation with agro-chemical companies.

Spray timing is likely to be critical for downy mildew control as its latent period is only a few days and frequent applications may be required to protect new growth. Programmes at 10 versus 14 days and at different timings in relation to infection events (protectant and curative), will identify weaknesses in product efficacy and persistence. Testing and ranking of fungicides will enable growers to select the best products for the range of diseases they encounter. The current choice of fungicides is limited and growers will require a selection of products to combine in programmes and minimise risks of selecting fungicide resistant strains. Results from this project will also be useful for product selection on other crops.

The specific objectives of the project were:

1. To confirm methods to ensure consistent development of lettuce downy mildew in screening experiments, using artificial inoculation with *Bremia lactucae* and to produce inoculum sufficient for use in objective 2.

2. To determine the crop safety and activity of novel and standard fungicides against lettuce downy mildew using a small-scale screening experiment.

3. To define the dose response activity of the most promising products.

4. To quantify the persistence of products, to guide timing of treatments.

Compliance

Three screening experiments were done in compliance with the requirements of the UK Official Recognition of Efficacy Testing scheme (ORETO). The protocols conformed to EPPO PP 1/65(3) (Downy mildews of lettuce and other vegetables) the exception being that the net plot size was twelve plants (rather than 30, suggested under glass) as these were preliminary screening experiments. Protocols conformed with EPPO PP1/35(3) (Guideline on phytotoxicity assessment) and PP1/152(3) (Guideline on design and analysis of efficacy evaluation trials). ADAS has Efficacy Testing Certificate No. ORETO 255.

Experiment 1: Efficacy and timing of fungicides against lettuce downy mildew

Introduction

Fungicide products selected in consultation with contacts in the agro-chemical industry and the grower coordinator, were screened in a replicated young plant test in an unheated polythene tunnel environment. The protectant and curative active of the test products was determined by applying fungicides prior to and after inoculation of lettuce seedlings with a spore suspension of *B. lactucae*, and subsequently providing environmental conditions favourable for downy mildew development.

Materials and methods

(Also see Appendix 1 – experiment diary)

Experiment design

The experiment comprised a two-way factorial design with twelve plants per plot (three pots of four plants) and four replicate blocks. There were 11 fungicide treatments applied at four different timings, with a full replication of the inoculated untreated control for each timing, to give a total of 48 treatments and 192 plots. A plot comprised three pots of four lettuce seedlings artificially inoculated with *B. lactucae*. Twelve extra pots each of four seedlings were placed away from the main trial area (to avoid infection via spore splash) as uninoculated untreated controls (not included in statistical analyses).

Plant material

Trays of lettuce seedlings (var. Frisco) in blocks at 2-3 true leaf stage were purchased from a commercial propagator. Variety Frisco is a cos-type lettuce that was known to be susceptible to infection by *B. lactucae* from previous observations.

Experimental layout

Seedlings that were healthy in appearance were transplanted into F2+S compost in 13 cm diameter pots, with four plants per pot (18 June). A plot comprised three pots placed on a layer of capillary matting within a gravel tray. The plots were raised up on rows of stacked chitting trays, to prevent rabbit damage. Pots for the uninoculated control were placed in a single chitting tray in a separate polytunnel.

Maintenance

Plants were maintained for approximately 1 week after transplanting until plants had reached the 3-4 true leaf stage, before commencing the experiment. Watering was to the capillary matting in the trays twice a day, rather than overhead watering. A Tiny tag data logger was used to record air temperature and relative humidity for the duration of the trial. Preventative biological pest control was used (*Aphidoletes*) for management of aphids.

Inoculation

On the day of inoculation (1 July), lettuce leaves with typical symptoms of downy mildew were collected from fresh and frozen stocks. The leaves were soaked in sterile distilled water and a sterile loop used to scrape the surface of the lesions to prepare a spore suspension of *B. lactucae*. The spore suspension was filtered through muslin and adjusted to a concentration of 2.5×10^4 spores/ml. A hand-held mister was used to spray all of the plants to the point of run-off (approximately 1 L spore suspension per block). After inoculation, the mypex matting between blocks was wetted and the trial area was covered with a ‘tent’ of clear polythene sheeting. The sheeting was left on for approximately 48 h (removed for the last spray application) to prolong leaf wetness duration and to promote high relative humidity. Uninoculated control plants were misted with water only and covered with separate sheeting.

Once polythene sheeting was removed, the plants were overhead misted 3 times per night, and hand watered twice per day (to the capillary matting).

Fungicide treatments

Fungicide treatments were applied either 5 days before (26 June), 2 days before (29 June), immediately prior to artificial inoculation (1 July), or 2 days after inoculation on 3 July. Fungicides were applied in 400 L water/ha (40 ml/m^2) using an Oxford precision sprayer with single nozzle (plus guard to prevent spray drift) at 2 Bar pressure. At each fungicide application, pots were taken to an adjacent polytunnel for treatment (to avoid spray drift) before replacing correctly in the trial layout. Treatments are shown in Table 1.

Table 1. Fungicide products included in Experiment 1

	Product	Active ingredient	Product rate / ha
1	Untreated control	-	-
2	Amistar	Azoxystrobin	1.0 L
3	Aliette 80 WG	Fosetyl-aluminium	3.0 kg
4	Fubol Gold	Mancozeb + metalaxyl-M	1.9 kg
5	Invader	Dimethomorph + mancozeb	2.0 kg
6	Previcur Energy	Fosetyl-aluminium + propamocarb hydrochloride	2.5 L
7	Revus	Mandipropamid	0.6 L
8	Signum	Boscalid + pyraclostrobin	1.5 kg
9	HDC F1	Not disclosed	2.5 L
10	HDC F2	Not disclosed	0.8 L
11	HDC F3	Not disclosed	2.5 kg
12	Valbon	Benthialvalicarb-isopropyl/mancozeb	1.6 kg

Notes:

Amistar	SOLA 1465/01 for outdoor lettuce
Aliette 80 WG	SOLA 3522/06 for outdoor lettuce
Fubol Gold	SOLA 2142/03 for outdoor lettuce
Invader	SOLA 3044/06 for outdoor lettuce
Previcur Energy	SOLA 0513/04 for outdoor lettuce
Revus	Administrative Experimental Approval COP 2009/00865
Signum	On-label for outdoor lettuce
HDC F1	Administrative Experimental Approval COP 2009/00865
HDC F2	Administrative Experimental Approval COP 2009/00865
HDC F3	Administrative Experimental Approval COP 2009/00865
Valbon	Administrative Experimental Approval COP 2009/00865

Assessments

The plants were assessed 8, 13, 15 and 21 days after inoculation. For each pot the following were recorded:

- Incidence (number of plants affected) and severity (% plant area affected) of downy mildew symptoms.
- Incidence of other diseases
- Incidence of any phytotoxicity, growth benefits or spray residues.

Statistical analyses

Data for phytotoxicity, disease incidence (proportion of plants per plot with symptoms) and disease severity (% plant area affected by symptoms) were analysed by ANOVA in Genstat.

Results and discussion

At 8 days after inoculation (9 July), there were symptoms of tip-burn and possible phytotoxicity. Tip-burn affected all treatments including the untreated control, though there were no significant effects of fungicides or timings. Symptoms that might be phytotoxicity was observed as scorch or yellow blotches on a few leaves. Again, incidence of symptoms was not specific to fungicide treatments and may not have been due to spray treatments. There was a lower incidence of phytotoxic symptoms on treatments that had received the final spray application (2 days after inoculation) (P<0.05), due possibly to cooler conditions at the time of fungicide application.

There were significant block effects on disease incidence and severity at both assessment times, with higher disease levels in block 4 (data not shown) corresponding with the right hand side of the polytunnel. The reason for the block difference is unclear, although environmental conditions may have been more favourable for disease development (lower temperatures, higher relative humidity) on this side of the polytunnel.

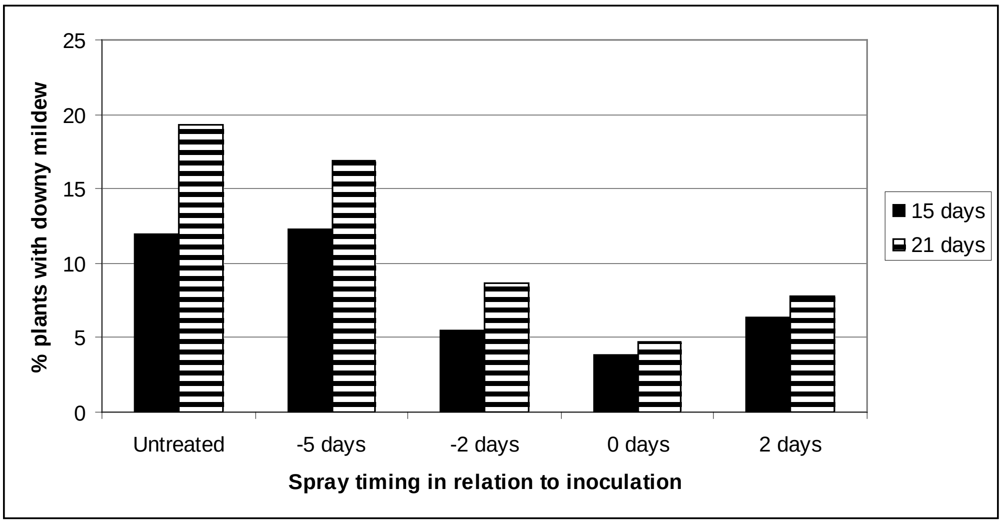


Figure 1. Effect of fungicide timing (mean of 11 products) on incidence of lettuce downy mildew, in comparison with an untreated control, at two intervals after inoculation in Experiment 1

Symptoms of downy mildew were first observed on 14 July, 13 days after inoculation. For the untreated control, downy mildew was recorded in 13 out of 16 plots. For the fungicide treatments, a maximum of 3 out of 16 plots were affected. Mean downy mildew incidence was 12% in the untreated control at 15 days after inoculation and had increased to 19% by 21 days after inoculation. There was a significant effect of timing at both assessments, irrespective of fungicide treatment. At 15 days, lower downy mildew incidence resulted from fungicide treatment at -2, 0 and +2 day timings compared with treatment 5 days before inoculation (*P*=0.006); a similar result was obtained when plants were assessed at 21 days after inoculation (*P*<0.001)(Figure 1). At 15 days, there was no effect of fungicides on downy mildew incidence (Table 2). There was no downy mildew in both the -2 and 0 day treatments of Invader and Signum (Table 2).

Table 2. Effect of fungicides on incidence of lettuce downy mildew, 15 days after inoculation in Experiment 1

Fungicide treatments		Mean % downy mildew incidence after spray timings at intervals before or after inoculation				Fungicide means
		- 5 days	- 2 days	0 days	+2 days	
1	Untreated control	-	-	-	-	12.0
2	Amistar	16.7	6.3	8.3	8.3	9.9
3	Aliette 80 WG	12.5	10.4	2.1	12.5	9.4
4	Fubol Gold	29.2	10.4	12.5	0.0	13.0
5	Invader	14.6	0.0	0.0	12.5	6.8
6	Previcur Energy	4.2	6.3	6.3	8.3	6.3
7	Revus	8.3	4.2	2.1	10.4	6.3
8	Signum	4.2	0.0	0.0	2.1	1.6
9	HDC F1	4.2	2.1	2.1	4.2	3.1
10	HDC F2	25.0	8.3	2.1	2.1	9.4
11	HDC F3	8.3	12.5	4.2	6.3	7.8
12	Valbon	8.3	0.0	2.1	4.2	3.7
Untreated vs fungicides (144 df)		Ns				
Fungicides only (144 df)		Ns				
Fungicides.timing (144 df)		Ns				

Table 3. Effect of fungicides on incidence of lettuce downy mildew, 21 days after inoculation in Experiment 1

Fungicide treatments		Mean % downy mildew incidence after spray timings at intervals before or after inoculation				Fungicide means
		- 5 days	- 2 days	0 days	+2 days	
1	Untreated control	-	-	-	-	19.3
2	Amistar	18.8	4.2	2.1	16.7	10.4
3	Aliette 80 WG	20.8	16.7	0.0	8.3	11.5
4	Fubol Gold	31.2	22.9	14.6	14.6	20.8
5	Invader	12.5	12.5	2.1	8.3	8.9
6	Previcur Energy	8.3	0.0	2.1	8.3	4.7
7	Revus	12.5	4.2	6.2	2.1	6.2
8	Signum	8.3	0.0	0.0	6.3	3.6
9	HDC F1	25.0	6.2	14.6	8.3	13.5
10	HDC F2	31.2	8.3	4.2	4.2	12.0
11	HDC F3	10.4	16.7	6.3	2.1	8.9
12	Valbon	6.3	4.2	0.0	6.2	4.2
SEDs (144 df):						
Untreated vs fungicides			3.64			

Fungicides only	4.92
Fungicide.timing	Ns

At 21 days, all of the fungicides except Fubol Gold had reduced downy mildew incidence by at least half compared with the untreated control. Fubol Gold gave similar levels of downy mildew incidence to the untreated control. Signum, Valbon and Previcur Energy were the best performing products, followed by Revus, Invader and HDC F3, all reducing disease incidence compared with Fubol Gold (*P*<0.029) and the untreated control (Table 3). There was no significant fungicide x timing interaction effect.

Disease severity remained low throughout the experiment (mean of <0.5% in the untreated control), due perhaps to high temperatures (Appendix 2). At 15 days after inoculation, there were no differences in disease severity due to fungicide treatments (data not shown). At this assessment, there was a timing effect (*P*<0.05), with lower disease severity due to fungicide timings at -2, 0 or +2 days, compared with -5 day timing and the untreated control. At 21 days after inoculation, there was no treatment timing effect. The fungicide treatments reduced disease severity overall compared with the untreated control (*P*<0.001), but there were no significant differences between the fungicides (Table 4).

Table 4. Effect of fungicide treatments on severity of lettuce downy mildew (averaged across fungicide timings), 21 days after inoculation in Experiment 1

	Fungicide treatments	Mean % plant area affected
1	Untreated control	0.43
2	Amistar	0.07
3	Aliette 80 WG	0.14
4	Fubol Gold	0.26
5	Invader	0.06
6	Previcur Energy	0.03
7	Revus	0.04
8	Signum	0.02
9	HDC F1	0.11
10	HDC F2	0.09
11	HDC F3	0.06
12	Valbon	0.02
	SEDs (144 df):	
	Untreated vs fungicides	0.086
	Fungicides only	Ns

Fungicides to include in the second experiment were selected largely on the basis of their effects on downy mildew incidence, since they could not be sufficiently differentiated using disease severity data. Previcur Energy, Revus, Signum and Valbon were selected as the most effective products. Invader and HDC F3 were similar to each other in efficacy, so Invader was selected for further experimentation since it is currently available for industry use. The poor performance of Fubol Gold (no different from the untreated control) in Experiment 1 may have been due to use of a strain of *B. lactucae* with resistance to metalaxyl-M.

Experiment 2: Defining the dose response activity of promising fungicides for the control of lettuce downy mildew

Introduction

A second inoculated experiment was done to define fungicide activity in relation to dose. Five products selected from Experiment 1 were evaluated to confirm efficacy and to identify the most effective products in relation to dose (using a screen at full and half dose) applied at four timings. The use of half dose treatments was done to help define the robustness of the treatments rather than to pursue low rates of application.

Materials and methods

(Also see Appendix 1 – experiment diary)

Experiment design

The experiment included five fungicide treatments each applied at full rate or half rate at one of four different timings, as well as a full replication of the inoculated untreated control and an inoculated water only control, to give a total of 48 treatments replicated in four blocks. A plot comprised three pots of four lettuce seedlings artificially inoculated with *Bremia lactucae*. Twelve extra pots each of four seedlings were placed away from the main trial area (to avoid infection via spore splash) as uninoculated untreated controls (not included in statistical analyses).

Plant material

Trays of lettuce seedlings (var. Frisco) in blocks at 2-3 true leaf stage were purchased from a commercial propagator.

Experimental layout

Seedlings that were healthy in appearance were transplanted into F2+S compost in 13 cm diameter pots, with four plants per pot (24 July). A plot comprised three pots placed on a layer of capillary matting within a gravel tray. The plots were raised up on rows of stacked chitting trays, to prevent rabbit damage. Pots for the uninoculated control were placed in a single chitting tray in a separate polytunnel.

Maintenance

Plants were maintained for approximately 1 week after transplanting until plants had reached the 3-4 true leaf stage, before commencing the experiment. Watering was to the capillary matting in the trays twice a day, rather than overhead watering. A Tiny tag data logger was used to record air temperature and relative humidity for the duration of the trial. Preventative biological pest control was used (*Aphidoletes*) for management of aphids. Caterpillar damage was observed on lettuces at the time of experiment set-up and so Hallmark Zeon (lambda-cyhalothrin) was applied at a rate of 0.08 L/ha in 1000L/ha water volume (24 July).

Inoculation

On the day of inoculation (29 July), lettuce leaves with typical symptoms of downy mildew were collected from fresh and frozen stocks. The leaves were soaked in sterile distilled water and a sterile loop used to scrape the surface of the lesions to prepare a spore suspension of *B. lactucae*. The spore suspension was filtered through muslin and adjusted to a concentration of 2.5 x 10^4 spores/ml. A hand-held mister was used to spray all of the plants to the point of run-off (approximately 1 L spore suspension per block). After inoculation, the mypex matting between blocks was wetted and the trial area was covered with a 'tent' of clear polythene sheeting. The sheeting was left on for approximately 48 h (removed for the last spray application) to prolong leaf wetness duration and to promote high relative humidity. Uninoculated control plants were misted with water only and covered with separate sheeting.

Once polythene sheeting was removed, the plants were overhead misted 3 times per night, and hand watered twice per day (to the capillary matting). Infector plants were placed in the paths between blocks to aid disease development.

Fungicide treatments

Table 5. Fungicide products included in Experiment 2

	Product	Active ingredient	Full product rate/ ha	Half product rate / ha
1	Untreated control	-	-	-
2	Water only control	-	-	-
3	Invader	Dimethomorph + mancozeb	2.0 kg	1.0 kg
4	Previcur Energy	Fosetyl-aluminium + propamocarb hydrochloride	2.5 L	1.25 L
5	Revus	Mandipropamid	0.6 L	0.3 L
6	Signum	Boscalid + pyraclostrobin	1.5 kg	0.75 kg
7	Valbon	Benthialvalicarb-isopropyl/mancozeb	1.6 kg	0.8 kg

Notes:

Invader	SOLA 3044/06 for outdoor lettuce
Previcur Energy	SOLA 0513/04 for outdoor lettuce
Revus	Administrative Experimental Approval COP 2009/00865
Signum	On-label for outdoor lettuce
Valbon	Administrative Experimental Approval COP 2009/00865

Fungicide treatments at either half or full rate were applied either 5 days before (24 July), 2 days before (27 July), immediately prior to artificial inoculation (29 July), or 2 days after inoculation on 31 July. Fungicides were applied in 400 L water/ha (40 ml/m^2) using an Oxford precision sprayer with single nozzle (plus guard to prevent spray drift) at 2 Bar pressure. At each fungicide application, pots were taken to an adjacent polytunnel for treatment (to avoid spray drift) before replacing correctly in the trial layout. Treatments are shown in Table 5.

Assessments

The plants were assessed 7, 12 and 16 days after inoculation. For each pot the following were recorded:

- Incidence (number of plants affected) and severity (% plant area affected) of downy mildew symptoms.
- Incidence of other diseases
- Incidence of any phytotoxicity, growth benefits or spray residues.

Statistical analyses

Data for disease incidence (proportion of plants per plot with symptoms) and disease severity (% plant area affected by symptoms) were analysed by ANOVA in Genstat.

Results and discussion

Disease development was more rapid in Experiment 2 compared with Experiment 1; symptoms of downy mildew were first observed on 5 August (7 days after inoculation). No symptoms of phytotoxicity were observed in this experiment.

Table 6. Effect of fungicide products and timing on incidence of downy mildew, 16 days after inoculation (averaged across doses) in Experiment 2

	Fungicide treatments	Mean % downy mildew incidence after spray timings at intervals before or after inoculation				Fungicide means
		- 5 days	-2 days	0 days	+ 2 days	
1	Untreated control	-	-	-	-	83.9
2	Water only control	66.7	87.5	68.7	77.1	75.0
3	Invader	43.8	77.1	65.6	79.2	66.4
4	Previcur Energy	91.7	61.5	70.8	75.0	74.7
5	Revus	69.8	64.6	71.9	76.0	70.6
6	Signum	86.5	76.0	67.7	88.5	79.7
7	Valbon	49.0	68.8	70.8	76.0	66.1
	Timing means (fungicides)	68.1	69.6	69.4	79.0	
SEDs (144 df):						
Untreated vs fungicides		5.80				
Fungicides only		4.73				
Fungicides.timing		9.47				

Disease incidence increased from 11% in the untreated control at 7 days after inoculation to 84% at 16 days after inoculation. At 16 days after inoculation, there was a significant fungicide x timing interaction (*P*<0.001), when disease incidence was averaged across doses (Table 6). This was due largely to a decrease in disease incidence when Invader and Valbon were applied 5 days before inoculation; this was in contrast to results from Experiment 1. There was also an increase in disease incidence when Previcur Energy was applied at this timing. Signum was more effective when applied at 0 days rather than at -5 or +2 days. There was no effect of fungicide dose on disease incidence.

Table 7. Effect of fungicide products and timing on severity of downy mildew, 16 days after inoculation in Experiment 2

	Fungicide treatments	Mean % downy mildew incidence after spray timings at intervals before or after inoculation				Fungicide means
		- 5 days	-2 days	0 days	+ 2 days	
1	Untreated control	-	-	-	-	3.1
2	Water only control	1.4	2.2	1.5	2.7	2.0
3	Invader	0.3	2.2	1.3	1.4	1.3
4	Previcur Energy	1.8	0.9	0.9	1.4	1.2
5	Revus	0.9	1.0	1.5	1.3	1.2
6	Signum	2.4	1.8	1.3	2.6	2.0
7	Valbon	0.6	1.7	1.5	2.2	1.5
	Timing means (fungicides)	1.2	1.5	1.3	1.8	
	SEDs (144 df):					
	Untreated vs fungicides	0.32				
	Fungicides only	0.26				
	Fungicides.timing	0.65				

Table 8. Effect of fungicide doses on severity of downy mildew, 14 days after inoculation in Experiment 2

	Fungicide treatments	% disease severity		Fungicide means
		Full dose	Half dose	
1	Untreated control	-	-	3.1
2	Water only control	-	-	2.0
3	Invader	1.8	0.8	1.3
4	Previcur Energy	1.3	1.2	1.2
5	Revus	1.1	1.3	1.2
6	Signum	1.9	2.2	2.0
7	Valbon	1.9	1.0	1.5
	Dose means	1.6	1.3	
	SEDs (144 df)			
	Fungicides.dose	0.37		

Disease severity in the untreated control was higher than that recorded in Experiment 1. At 16 days after inoculation, there was a significant fungicide x timing interaction (*P*=0.007) (Table 7). As with disease incidence, this was due largely to a decrease in disease incidence when Invader and Valbon were applied 5 days before inoculation. In addition, Signum was more effective when applied at 0 days rather than at -5 or +2 days. There was a significant interaction effect of fungicide x dose on disease severity (*P*=0.046) (Table 8). Invader and Valbon were more effective when applied at half dose, while Previcur Energy, Revus and Signum gave similar levels of control at full and half dose.

Experiment 3: Quantifying the persistence of fungicides for control of lettuce downy mildew, to guide spray timing

Introduction

A third inoculated experiment was done to define the persistence of disease control in relation to timing of fungicide programmes (two sprays at either 10 or 14 day intervals) for the most promising five products. This experiment involved re-inoculation so providing a discrete targeted challenge of persistence, rather than more general exposure to spores during the course of the experiment.

Materials and methods

(Also see Appendix 1 – experiment diary)

Experiment design

The experiment included five fungicide treatments (full rate), applied on the day of 1^{st} inoculation (day 0) and either 10 days later (before 2^{nd} inoculation) or 14 days later. There was a full replication of the inoculated untreated control and an inoculated water only control, to give a total of 14 treatments replicated in four blocks. A plot comprised three pots of four lettuce seedlings artificially inoculated with *B. lactucae*. Twelve extra pots each of four seedlings were placed away from the main trial area (to avoid infection via spore splash) as uninoculated untreated controls (not included in statistical analyses).

Plant material

Trays of lettuce seedlings (var. Frisco) in blocks at 2-3 true leaf stage were purchased from a commercial propagator.

Experimental layout

Seedlings that were healthy in appearance were transplanted into F2+S compost in 13 cm diameter pots, with four plants per pot (20 August). A plot comprised three pots placed on a layer of capillary matting within a gravel tray. The plots were raised up on rows of stacked chitting trays, to prevent rabbit damage. Pots for the uninoculated control were placed in a single chitting tray in a separate polytunnel.

Maintenance

Plants were maintained for approximately 1 week after transplanting until plants had reached the 3-4 true leaf stage, before commencing the experiment. Watering was to the capillary matting in the trays twice a day, rather than overhead watering. A Tiny tag data logger was used to record air temperature and relative humidity for the duration of the trial. Preventative biological pest control was used (*Aphidoletes*) for management of aphids. In addition, *Diglyphus* were released for leaf miner control as leaf damage due to this pest had been observed. Caterpillar damage was observed on lettuces at the time of experiment set-up and so Dipel WF (*Bacillus thuringiensis*) was applied at a rate of 1 L/ha in 1000L/ha water volume (27 August).

Inoculation

On the day of the 1st inoculation (25 August), lettuce leaves with typical symptoms of downy mildew were collected from fresh and frozen stocks. The leaves were soaked in sterile distilled water and a sterile loop used to scrape the surface of the lesions to prepare a spore suspension of *B. lactucae*. The spore suspension was filtered through muslin and adjusted to a concentration of 3 x 10^4 spores/ml. A hand-held mister was used to spray all of the plants to the point of run-off (approximately 1 L spore suspension per block). After inoculation, the mypex matting between blocks was wetted and the trial area was covered with a 'tent' of clear polythene sheeting. The sheeting was left on for approximately 48 h (removed for the last spray application) to prolong leaf wetness duration and to promote high relative humidity. Uninoculated control plants were misted with water only and covered with separate sheeting.

Once polythene sheeting was removed, the plants were overhead misted 3 times per night, and hand watered twice per day (to the capillary matting). Infector plants in trays were placed in pathways between each block to aid disease development. Frequency of hand watering was reduced later in the experiment as temperatures dropped.

A 2nd inoculation was applied 10 days after the 1st inoculation (4 September), using 1 x 10^5 spores / ml using the same method as described previously. The plants were covered with polythene for 24 h.

Fungicide treatments

Fungicide treatments at full rate were applied on days 0 and 10 (25 August, 4 September) or days 0 and 14 (25 August, 8 September). Sprays on days 0 and 10 were applied immediately prior to the 1st and 2nd inoculation, respectively. Fungicides were applied in 400 L water/ha (40 ml/m^2) using an Oxford precision sprayer with single nozzle (plus guard to prevent spray drift) at 2 Bar pressure. At each fungicide application, pots were taken to an adjacent polytunnel for treatment (to avoid spray drift) before replacing correctly in the trial layout. Details of fungicide products are shown in Table 9.

Table 9. Fungicide products included in Experiment 3

	Fungicide	Product rate / ha
1	Untreated control	-
3	Water only control	-
5	Invader	2.0 kg
7	Previcur Energy	2.5 L
9	Revus	0.6 L
11	Signum	1.5 kg
13	Valbon	1.6 kg

Notes:

Invader	SOLA 3044/06 for outdoor lettuce
Previcur Energy	SOLA 0513/04 for outdoor lettuce
Revus	Administrative Experimental Approval COP 2009/00865
Signum	On-label for outdoor lettuce
Valbon	Administrative Experimental Approval COP 2009/00865

Assessments

The plants were assessed on the following days after inoculation: 10, 14, 20, 27, 30, 34 and 37 days. For each pot the following were recorded:

- Incidence (number of plants affected) and severity (% plant area affected) of downy mildew symptoms.
- Incidence of other diseases
- Incidence of any phytotoxicity, growth benefits or spray residues.

Statistical analyses

Data for disease incidence (proportion of plants per plot with symptoms) and disease severity (% plant area affected by symptoms) were analysed by ANOVA in Genstat.

Results and discussion

At the 10 day spray, Revus was applied instead of Signum, and vice versa, in error; all other spray applications were correct. The data for these plots was included in statistical analyses and is shown in the results as Revus / Signum, or Signum / Revus programmes.

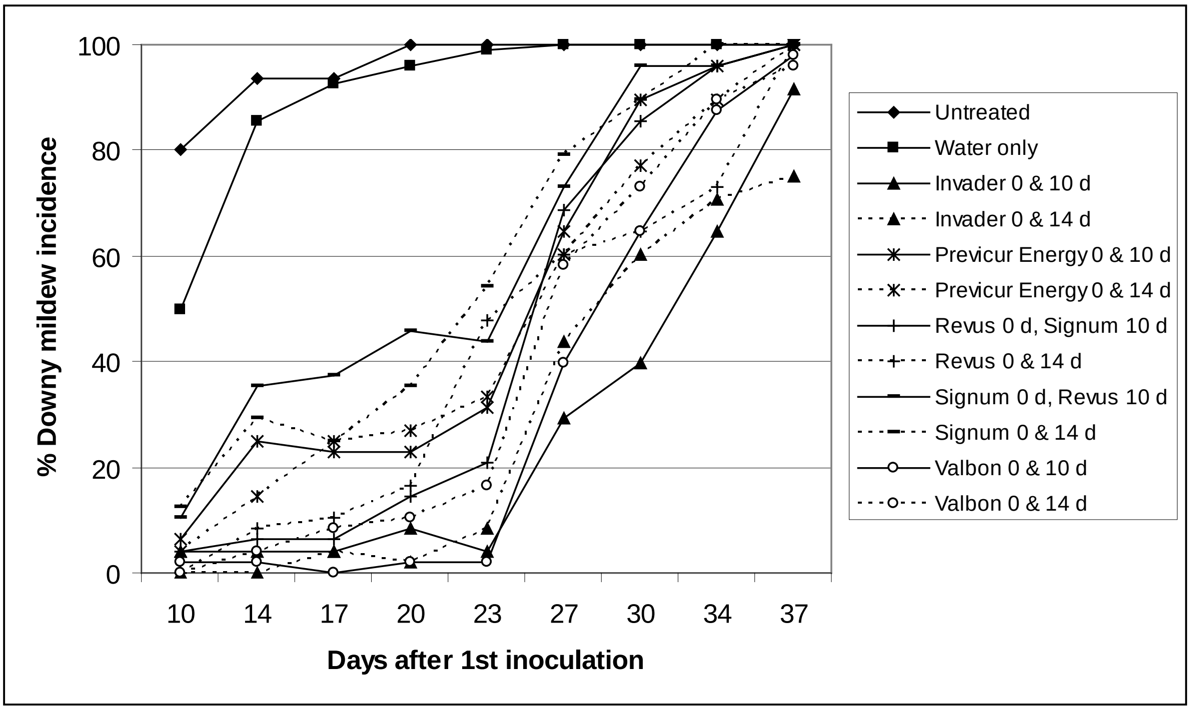
Disease development was rapid with 71% downy mildew incidence by 10 days after 1st inoculation and all plants affected at 20 days, in the untreated control (Figure 2). At 20 days, disease incidence was less than 50% for all of the fungicide treatments compared with 100% in the untreated control (Table 10). Up until 27 days after inoculation, all of the fungicides significantly reduced disease incidence ($P<0.001$) in comparison with the untreated and water controls. At 34 days, disease incidence was still significantly lower than the controls for the two Invader programmes and the Revus programme at 0 & 14 days. Irrespective of fungicide timing, the Previcur Energy treatments, and programmes commencing with

Signum were less effective in reducing downy mildew incidence at 14 and 20 days after inoculation compared with Invader, Revus (and Signum), and Valbon. Invader was the most effective product over the duration of the experiment. Invader was also the only product for which a spray interval of 10 days gave better control than a 14 day interval; for the other products, downy mildew incidence did not vary significantly with spray timing interval.

Table 10. Effect of fungicide timing intervals on incidence of downy mildew in Experiment 3

	Fungicides	Timing	% downy mildew incidence at intervals after inoculation			
			14 days	20 days	27 days	34 days
1	Untreated control	-	93.8	100.0	100.0	100.0
2	Water control	-	85.4	95.8	100.0	100.0
3	Invader x 2	0 & 10 days	4.2	8.3	29.2	64.6
4	Invader x 2	0 & 14 days	0.0	2.1	43.7	70.8
5	Previcur Energy x 2	0 & 10 days	25.0	22.9	64.6	95.8
6	Previcur Energy x 2	0 & 14 days	14.6	27.1	60.4	89.6
7	Revus/Signum	0 & 10 days	6.3	14.6	68.7	95.8
8	Revus x 2	0 & 14 days	8.3	16.7	60.4	72.9
9	Signum/Revus	0 & 10 days	35.4	45.8	72.9	95.8
10	Signum x 2	0 & 14 days	29.2	35.4	79.2	100.0
11	Valbon x 2	0 & 10 days	2.1	2.1	39.6	87.5
12	Valbon x 2	0 & 14 days	4.2	10.4	58.3	89.6
	SED (41 df)					
	Controls vs fungicides		5.89	6.30	9.13	9.06
	Fungicides		6.80	7.27	10.54	10.47

Figure 2. Effect of fungicides on incidence of downy mildew at intervals after 1st inoculation in Experiment 3



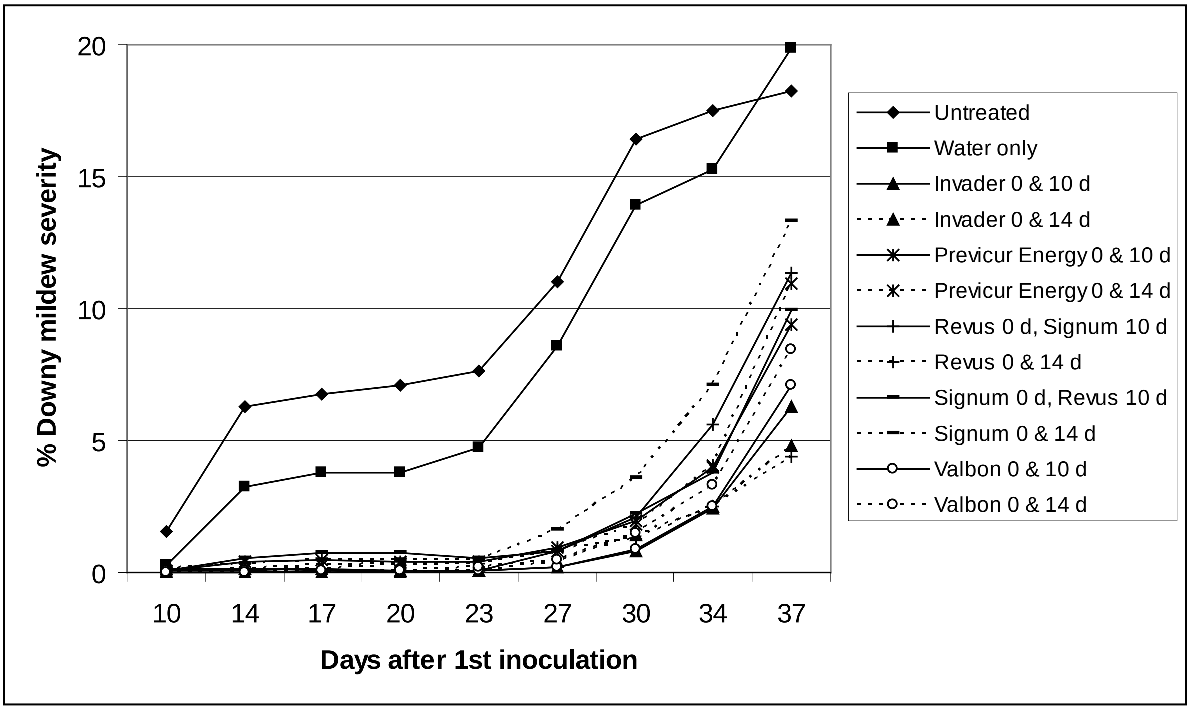
Note: 2^{nd} inoculation at 10 days after 1^{st} inoculation

Disease severity increased to 18% of leaf area affected at 34 days after the 1st inoculation compared with 7% or less in the fungicide treatments (Table 11, Figure 3). The fungicide treatments significantly reduced disease severity in comparison with the controls at all of the assessment dates shown in Table 11. Up until 27 days, disease severity remained low (1.6% or less), irrespective of fungicide product or spray intervals. At 34 days, disease severity was lower for the Invader programmes and Valbon programmes compared with Signum (0 & 14 days) (Figure 3).

Table 11. Effect of fungicide timing intervals on severity of downy mildew in Experiment 3

	Fungicides	Timing	% downy mildew incidence at intervals after inoculation			
			14 days	20 days	27 days	34 days
1	Untreated control	-	6.3	7.1	11.0	17.5
2	Water control	-	3.3	3.8	8.6	15.3
3	Invader x 2	0 & 10 days	0.2	0.1	0.2	2.4
4	Invader x 2	0 & 14 days	0.0	0.0	0.4	2.5
5	Previcur Energy x 2	0 & 10 days	0.4	0.4	0.9	4.0
6	Previcur Energy x 2	0 & 14 days	0.2	0.3	0.8	4.0
7	Revus/Signum	0 & 10 days	0.0	0.0	0.8	5.6
8	Revus x 2	0 & 14 days	0.1	0.3	0.9	2.5
9	Signum/Revus	0 & 10 days	0.6	0.7	0.8	3.8
10	Signum x 2	0 & 14 days	0.3	0.5	1.6	7.1
11	Valbon x 2	0 & 10 days	0.1	0.1	0.2	2.5
12	Valbon x 2	0 & 14 days	0.0	0.1	0.5	3.3
	SED (41 df)					
	Controls vs fungicides		0.93	0.90	1.10	1.51
	Fungicides		1.08	1.03	1.27	1.75

Figure 3. Effect of fungicides on severity of downy mildew at intervals after 1st inoculation in Experiment 3



Note: 2nd inoculation at 10 days after 1st inoculation

Analysis of disease incidence and severity data for the different treatments indicated that, except for Invader, levels of downy mildew control were similar whether 10 day or 14 day spray intervals were used. However, from looking at Figures 2 and 3, it is apparent that the rate of disease development on fungicide-treated plants was generally more rapid from day 20 (after 1st inoculation) for incidence and from day 23 for severity. New symptoms at day 23 would indicate infection took place 5-7 days previously. This is further support for the duration of control being not more than 10 days. Therefore, in a field situation under high disease pressure, programmes with 7-10 day spray intervals may be required.

This project has identified some promising products with subtle differences in performance relating to timing. Further work is required to confirm these effects as some differences in product rankings were evident between the three experiments. Valbon and Invader worked well when applied 5 days before inoculation whereas Signum was most effective at Day 0 in Experiment 2. Such effects may become more important if infection periods can be forecasted and used to guide spray applications. Valbon and Invader showed significant dose effects which merit further examination to define full dose-response curves. The differences in control between half and full dose for Valbon and Invader remain unexplained. For both products the control achieved with full rate was poorer at timings -2 and + 2 days than at -5 and 0 days. Only a limited selection of products were examined in the three experiments but some candidates used in Experiment 1 clearly have useful activity against downy mildew and should be examined in future projects.

The next stage to exploit these results is to optimise product selection and timing under field conditions. This will need to consider the range of diseases affecting individual crops (e.g .botrytis, sclerotinia, rhizoctonia bottom rot and ringspot), the spectrum of activity of the available fungicides and diversification to minimise risks of fungicide resistance.

Conclusions

Fungicide efficacy and timing

- Of 11 fungicide products evaluated initially for control of downy mildew (*B. lactucae*) on lettuce in Experiment 1, the following were most effective in reducing disease incidence: Previcur Energy, Revus, Signum and Valbon. Invader and coded product HDC F3 were moderately effective.
- There was a significant effect of timing, irrespective of fungicide treatment. Lower downy mildew incidence resulted from fungicide treatment at -2, 0 and +2 day timings compared with treatment at 5 days before inoculation.
- Fubol Gold did not reduce disease incidence in comparison with the untreated control, potentially due to use of a strain of *B. lactucae* with resistance to metalaxyl-M.

Fungicide dose

- In Experiment 2, the five fungicides tested (Invader, Previcur Energy, Revus, Signum and Valbon) again reduced disease incidence and severity in comparison with the untreated control.
- Disease incidence was not affected by halving fungicide dose. For disease severity, Invader and Valbon were more effective when applied at half dose, while efficacy of Previcur Energy, Revus and Signum was unaffected when the dose was halved.

Fungicide persistence

- When the same five fungicides were applied at spray intervals of 10 and 14 days (with 2 inoculation events) in Experiment 3, all products reduced downy mildew incidence to <50% for up to 20 days (compared with 100% incidence in the untreated control). Invader was the most effective product followed by Revus and Valbon.
- While spray intervals of 10 days were generally no less effective than 14 day intervals in this experiment, examination of disease progress curves suggested that in a field situation under high disease pressure, 10 day intervals are likely to be more robust.

Technology transfer

A presentation on the project has been requested at the conference of the British Leafy Salads Association, November 24 2010, and an 'HDC News' article will be provided.

Acknowledgements

Provision of fungicides by agro-chemical companies is gratefully acknowledged.

Appendix 1. Experiment diaries

Experiment 1: Efficacy and timing of novel and standard fungicides against lettuce downy mildew (ADAS Boxworth, spring 2009)

Trial Diary

Date	Task
	Preliminary inoculation studies:
30/3/2009	Lettuce cv. Corsa from propagator sown.
27/4/2009	Lettuce inoculated with *Bremia lactucae* spore suspension of 3 x 10^5 cfu per ml. from frozen leaves of lettuce cv. Chartwell ex Bisworth (21/10/08). Plants placed into controlled environment cabinet at 15^0C, 12h day/12h night. 100mls of spore suspension frozen for later use.
29/4/2009	Inoculated lettuce removed from CE cabinet and placed in polytunnel. Uncovered by day, leaves misted and re-covered overnight for 8 days.
8/5/2009	No disease seen so lettuce re-inoculated with defrosted spore suspension of 3 x 10^5 cfu per ml. *Bremia lactucae*. Uncovered by day, leaves misted and re-covered overnight for 11 days.
19/5/2009	Downy mildew seen on lettuce. Aphids observed, Aphox applied to run off.
22/5/2009	Lettuce transplanted from plugs to pots.
27/5/2009	Aphidoletes applied to further control aphids.
1/6/2009	Leaves removed and spore suspension of *Bremia lactucae* made-up and then frozen for later use on main trial. Lettuce sown at propagators.
12/6/2009	Leaves with downy mildew picked and frozen for later use on trial.
	Main experiment:
18/6/2009	Lettuce from propagators cv. Frisco transplanted out into 600 13cm pots of F2 + S compost to give 12 plants per plot (3 pots x 4 plants). Lettuce raised to avoid pest damage. Trial set out in polytunnel as per protocol, watering twice daily into trays. Spare lettuce inoculated and placed in a separate polytunnel. Spare lettuce misted at night to encourage sporulation.
26/6/2009	1st spray applied. (-5 days)
29/6/2009	2nd spray applied. (-2 days)
1/7/2009	3rd spray applied. (0 days) Lettuce inoculated with *Bremia lactucae* spore suspension of 2.5 x 10^4 cfu per ml. 1L of spore suspension applied per block. Approx 20mls per plot. Trial covered.
3/7/2009	Trial uncovered. 4th spray applied. (+2 days).
10/7/2009	Misting at night by overhead irrigation initiated to encourage sporulation and disease alongside continued watering into trays.
14/7/2009	First symptoms of disease seen. 1st disease and phytotoxicity assessments completed at 13 days.
16/7/2009	15 day disease assessment completed.
21/7/2009	Nutrient feed applied to trial.
22/7/2009	21 day disease assessment completed.
23/7/2009	Logger downloaded. Trial finished.

Experiment 2: Defining the dose response activity of promising fungicides for the control of lettuce downy mildew (ADAS Boxworth, summer 2009)

Trial Diary

Date	Task
17/7/2009	Lettuce from propagators cv. Frisco transplanted out into 600 13cm pots of F2 + S compost to give 12 plants per plot (3 pots x 4 plants). Plants placed into greenhouse and watered gently overhead.
23/7/2009	Downy mildew affected lettuce leaves from untreated plots in trial 1 removed and frozen for later use on current trial. Remaining downy mildew affected lettuce from untreated plots moved into separate polytunnel, to be later used as infector plants.
24/7/2009	Trial set up as per protocol in polytunnel and raised to avoid pest damage. Watering twice daily into trays. 1st spray applied. (-5 days) Caterpillar damage seen on trial and Hallmark Zeon applied at a rate of 0.08L/ha at 1000L/ha water volume.
27/7/2009	2nd spray applied. (-2 days)
29/7/2009	3rd spray applied. (0 days) Lettuce inoculated with *Bremia lactucae* spore suspension of 2.5 x 10^4 cfu per ml. 1L of spore suspension applied per block. Approx 20mls per plot. Trial covered.
31/7/2009	Trial uncovered. 4th spray applied. (+2 days).
1/8/2009	Infector plants in trays placed into paths between each block to aid disease development.
3/8/2009	Misting at night by overhead irrigation initiated to encourage sporulation and disease alongside continued watering into trays.
5/8/2009	First symptoms of disease seen. 1st disease and phytotoxicity assessments completed at 7 days. No phytotoxicity.
10/8/2009	2nd disease assessment completed at 12 days.
14/8/2009	3rd disease assessment completed at 16 days.
17/8/2009	Logger downloaded. Trial finished.

Experiment 3: Quantifying the persistence of fungicides for control of lettuce downy mildew, to guide spray timing (ADAS Boxworth, summer/autumn 2009)

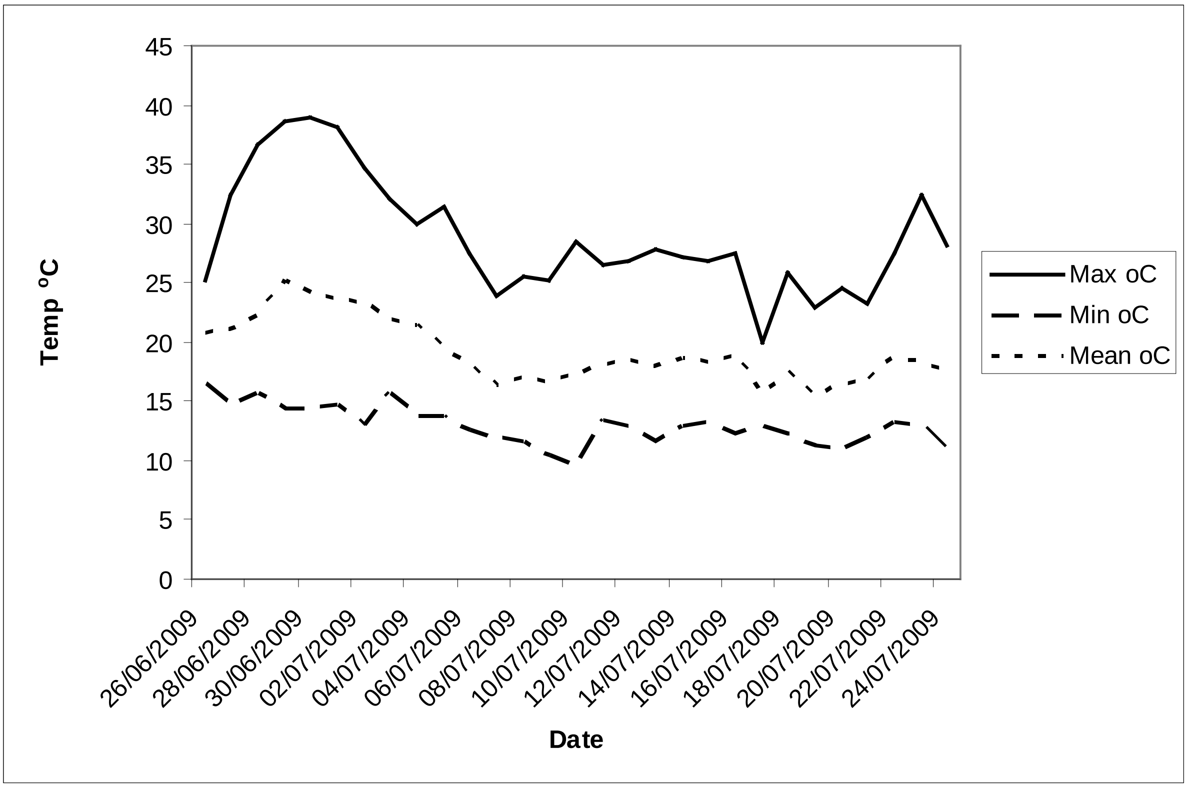
Trial Diary

Date	Action carried out
17/8/2009	Remaining downy mildew affected lettuce from untreated plots from trial 2 moved into separate polytunnel, to be later used as infector plants.
20/8/2009	Lettuce from propagators cv. Frisco transplanted out into 200 13cm pots of F2 + S compost to give 12 plants per plot (3 pots x 4 plants). Trial set up as per protocol in polytunnel and raised to avoid pest damage. Watering daily into trays.
21/8/2009	Downy mildew affected lettuce leaves from untreated plots in trial 2 removed and used to make *Bremia lactucae* spore suspension at 4 x 10^4 cfu per ml. 250mls used to inoculate 40 pots of lettuce to be used later in the trial to make further inoculum. Plants placed into polythene bags in controlled environment cabinet at 15^{0}C, 12h day/12 h night.
25/8/2009	'Inoculator' plants removed from CE cabinet and placed in separate polytunnel. 1st spray applied. (0 days) Lettuce inoculated with *Bremia lactucae* spore suspension of 3 x 10^4 cfu per ml. 0.5 L of spore suspension applied per block. Approx 35 ml per plot. Trial covered.
27/8/2009	Caterpillar damage seen on trial and Dipel WF applied at a rate of 1L/ha at 1000L/ha water volume. Leaf miner damage also seen. *Diglyphus* and *Aphidoletes* released for leaf miner and aphid control.
1/9/2009	Infector plants in trays placed into paths between each block to aid disease development.
2/9/2009	First symptoms of downy mildew seen on untreated controls.
3/9/2009	1st disease and phytotoxicity assessment completed at 9 days. No phytotoxicity seen.
4/9/2009	2nd spray applied. (+10 days). T9 and T11 sprays applied to wrong plots. Lettuce inoculated with *Bremia lactucae* spore suspension of 1 x 10^5 cfu per ml made up from 'inoculator' plants. 0.5 L. Trial covered. Trial uncovered after 24 hrs.
6/9/2009	3rd spray applied. (+14 days).
8/9/2009	2nd disease assessment completed at 14 days. 3rd disease assessment completed at 17 days.
11/9/2009	Misting at night by overhead irrigation initiated to encourage sporulation and disease alongside continued watering into trays. Watering into trays now 2-3 times a week as weather cools. Nutrient feed applied to trial. 4th disease assessment completed at 20 days.
14/9/2009	5th disease assessment completed at 23 days. Nutrient feed applied to trial.
17/9/2009	6th disease assessment completed at 27 days. 7th disease assessment completed at 30 days.
21/9/2009	8th disease assessment completed at 34 days.
24/9/2009	9th disease assessment completed at 37 days.
28/9/2009	Logger downloaded. Trial finished.
1/10/2009	

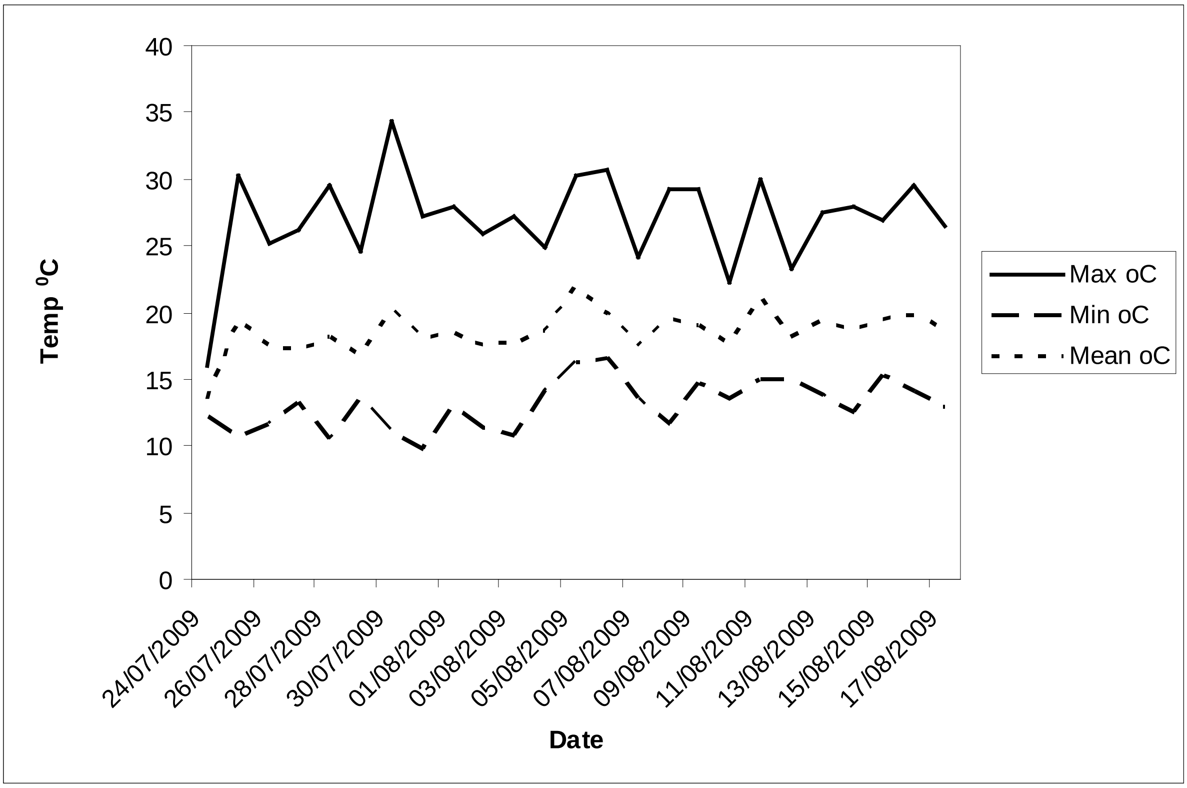
Appendix 2. Temperature data for Experiments 1-3

Polythene tunnel, ADAS Boxworth, Cambs

Experiment 1



Experiment 2



Experiment 3