

Project title: Protected Lettuce: Evaluation of novel fungicides and fungicide programmes for the control of downy mildew (*Bremia lactucae*).

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Project leader: Dr G M McPherson

Location: Horticulture Research International  
Stockbridge House, Cawood,  
Selby, North Yorkshire, YO8 0TZ  
Tel: 01757 268275. Fax: 01757 268996.

Project co-ordinator: Mr D Hargreaves

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The results and conclusions in this report are based on a series of three experiments. The conditions under which the experiments were carried out and the results have been reported with detail and 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.

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## **PRACTICAL SECTION FOR GROWERS**

### **Objectives and Background**

Downy mildew, caused by the fungus *Bremia lactucae* continues to be one of the primary disease problems in protected lettuce. The occurrence of novel strains of the fungus resistant to metalaxyl in the early 1980's has made control of the disease with fungicides difficult.

A unique strategy utilising the Dm or R-genes in the various lettuce cultivars in conjunction with continued use of metalaxyl has been adopted widely and successfully by the lettuce industry. However, occasionally reports of control failure due to the occurrence of a new strain of *Bremia* pathogenic on the cultivars which had previously conferred resistance, have been received. It is likely to be only a matter of time before a strain of *Bremia* resistant to metalaxyl and which has virulence to all the currently used Dm or R-genes in lettuce cultivars occurs. It is essential therefore that alternative fungicides are sought which are effective against this pathogen and which are safe to the crop, the consumer and the environment.

Already good progress has been made in this area. Both Aliette and Filex now have Specific Off-Label Approval for use on lettuce and this has assisted growers in maintaining control. However, these products are limited in their use. Occasional reports of phytotoxicity following the use of Aliette have been received though never fully substantiated. In addition, it has a restricted use pattern towards crop maturity. Filex in contrast can be used later towards crop maturity though tends to be less effective than Aliette particularly when disease pressure is high.

The objective of this project was to evaluate a range of novel fungicides with claimed activity against oomycete pathogens with a view to securing On or Off-Label Approval for use on lettuce in order to complement the current products and to further minimise the risk of resistance development.

## Summary of Results

Over a four year period (1993-1996) three fully replicated trials were conducted on butterhead lettuce to determine the relative efficacy of a range of novel fungicides and fungicide programmes against *Bremia lactucae*, cause of downy mildew, following artificial inoculation. Work originally planned for 1995 was postponed until 1996 due to the unavailability of important novel fungicides in that year.

In all trials downy mildew established successfully in the experimental crop area and provided a series of stern tests for the fungicides selected for study. The apparent influx of natural metalaxyl resistant variants in the population in some years affected the performance of the standard product based on metalaxyl (Favour 600SC).

In 1993, the standard dithiocarbamate fungicide mancozeb was moderately effective in the early stages of the trial and was superior to thiram. However, the level of *Bremia* control in these dithiocarbamate treatments declined towards crop maturity due primarily to the restrictions on application timings imposed on these products. The standard systemic fungicide metalaxyl (in Favour 600 SC) was effective against the metalaxyl sensitive strain of *Bremia* introduced into the trial area though a relatively high incidence of plant infection in this treatment at maturity suggested that one or more metalaxyl resistant strains had occurred in the crop naturally. This highlights one of the main problems facing lettuce growers in formulating an effective disease control programme on the crop.

The integrated programmes based around Aliette block incorporation, Aliette drenches (SOLA recommendation), Filex (SOLA) or Filex + Mancozeb were very effective providing excellent disease suppression throughout the first years trial and yielding the highest head weights at harvest, albeit with an increased number of fungicide applications.

Of the novel fungicides trialled in 1993, fluazinam (Shirlan) was phytotoxic to young lettuce plants and further evaluation with this product ceased. Three potato 'blight' fungicides (Guardian, Fytospore and Trustan) with novel active ingredients were expected to provide good control of *Bremia* yet were largely disappointing. Only Trustan provided a moderate reduction in downy mildew and a marked increase in head weight. As a result of the evaluation it was concluded that none of the novel fungicides evaluated offered improved performance compared to those products already approved (On or Off-Label) on lettuce.

The most effective treatment regime in 1993 was based on Aliette block incorporation in combination with Filex applied according to the Specific Off-Label Approval and this provided a 64% increase in trimmed head weight (see Annual Report for 1993 for more details).

In autumn 1994 a second trial was conducted using a range of different novel treatments as they became available. Again, a metalaxyl sensitive strain of *Bremia lactucae* was introduced and a significant loss in head weight occurred due to leaf infection by this pathogen. The standard fungicide Favour 600SC provided effective control in 1994 and, unlike in 1993, there was little evidence of metalaxyl insensitive strains occurring in the population to cause control failure.

Due to the anticipated withdrawal of products containing zineb\* (including zineb dust), alternative dithiocarbamate products (Mancozeb 8% dust, Mancozeb 15% dust, Mancozeb 80% WP) were evaluated in 1994 to determine whether an equivalent level of control could be achieved. Both mancozeb dust formulations and mancozeb 80% WP provided control of *Bremia* equivalent to zineb and there was some evidence, in contrast to popular opinion, that this formulation was more effective than the dust formulation. Where all these protectant products were applied control deteriorated after the last application. Due to the restrictions imposed on their use post-planting (two applications within two weeks of planting) protectant dithiocarbamate fungicides clearly cannot be relied upon to maintain control through to

harvest. Alternative products with shorter harvest intervals are clearly required to maintain protection through to crop maturity. In this respect, an integrated programme, albeit complex, based on Aliette and Mancozeb WP and Filex post-planting did provide effective control through to harvest and yielded the highest head weight increase (31%) in this 1994 evaluation.

**\* Note: The outcome of the EU dithiocarbamate review is still not available and at the time of writing this report Zineb WP was still available for use on lettuce.**

Two further novel fungicides, Invader (dimethomorph + mancozeb) and Tattoo (propamocarb hydrochloride + mancozeb) were included in the 1994 experiment and, whilst they appeared to provide moderate control of *Bremia*, they were limited in their effectiveness because of the dithiocarbamate partner in the mixture; their application being restricted to two treatments within two weeks post-planting. Quite clearly, the decision to incorporate a protectant dithiocarbamate fungicide in the formulation of novel 'blight' fungicides, whilst useful in anti-resistance strategies in potatoes; hinders their potential in horticultural crops such as lettuce.

No evaluations were conducted in 1995 because the anticipated arrival of both the new strobilurin fungicides and the new 'Plant Activator' from Novartis was delayed and, following agreement with HDC the project was postponed until they became available for evaluation in 1996. Fortunately these products were made available in time for a further evaluation in autumn 1996 and these results are presented in this report.

As in 1993 the performance of Favour 600SC in 1996 declined during the time course of the study and it was assumed that metalaxyl resistant strains had occurred naturally in the population, adversely affecting the performance of this product. The dithiocarbamate mancozeb performed well though was again let down by its short persistence after the last application two weeks post-planting. As in 1994, the Mancozeb 80% WP was superior to the equivalent dust formulation and this dispels popular opinion that the dust formulation is preferable because it avoids wetting the foliage, thereby exacerbating the disease. It is

recognised however that many growers, particularly propagators, prefer the convenience of dust applications and the loss of Zineb dust has created some difficulty.

Unfortunately, dust formulations of Mancozeb dust are not currently approved for use in the UK and on the basis of the results obtained during this study it will be extremely difficult to justify pursuing them.

Two integrated programmes were evaluated alongside each other in 1996. One based on Aliette (Integrated programme 2) gave significantly better control than one based on Filex (Integrated programme 1). This was further supported by the good control achieved using Aliette applied alone according to the Specific Off-Label Approval (SOLA No. 0223/95). The best integrated programme (2) increased the mean trimmed lettuce head weight by 106% in this evaluation from 122 g to 251 g/plant.

The use of a cellulose spray additive in conjunction with Aliette was also included in the 1996 evaluation as it had been claimed that disease control could be improved with this product. However, no evidence was gained in this trial to substantiate this.

The new strobilurin fungicide azoxystrobin (Amistar) from Zeneca was included for the first time and provided a moderate suppression of *B. lactucae*. This fungicide is broad spectrum in action and has now been demonstrated in other HDC funded studies to be effective against *Botrytis* in lettuce, together with *Sclerotinia* and *Rhizoctonia* in celery. It is also claimed to have good activity against powdery mildews and leaf-spot fungi such as *Septoria* and *Phoma* in various crops. In this respect, azoxystrobin has potential in many horticultural crops, including lettuce. Currently however, the only approved UK use for this product is on cereals though discussions are underway with the manufacturers with a view to progressing On or Off-Label Approval on various horticultural crops, including lettuce. It is anticipated that some additional residue data will be required on lettuce and hopefully this can be progressed via the HDC sponsored SOLA programme, preferably in collaboration with the manufacturers.



The new 'Plant Activator' from Novartis (formerly Ciba-Geigy) was extremely poor in its first years evaluation. Indeed, there was slight evidence that it actually exacerbated *Bremia* infection on the crop. Discussions are taking place with Novartis to find a satisfactory explanation for this phenomenon.

Finally, the potato blight fungicide Invader was again unable to provide satisfactory control of *B. lactucae* in its second year evaluation. There appears to be little merit therefore in pursuing its use on protected lettuce where dithiocarbamate restrictions limit its use post-planting.

In conclusion, this three year study has highlighted:

1. The vulnerability of mildew control programmes which rely on metalaxyl, where Dm or R-genes are not utilised.
2. The good protectant activity of Mancozeb 80% WP and the equivalent dust formulations.
3. The excellent control of *Bremia*, under high disease pressure, using integrated programmes based on Aliette and Filex, but particularly the former product.
4. The moderate control of *Bremia* using the new strobilurin fungicide Amistar. Whilst not performing particularly outstandingly against *Bremia*, this product could have tremendous potential in reducing the fungicide burden on the lettuce crop due to its wide ranging, broad spectrum activity, assuming an On or Off-Label Approval can be secured for its use on lettuce in the UK.

## Action Points for Growers

- \* Growers, where possible, should continue to utilise the Dm or R-genes in lettuce cultivars in conjunction with metalaxyl to control downy mildew.
- \* Growers should recognise the risk associated with the use of metalaxyl where host resistance is not available.
- \* The dithiocarbamate fungicide mancozeb, applied as a WP formulation should be used particularly during propagation to protect plants from *Bremia* infection.
- \* Growers should consider adopting an integrated strategy based on Aliette or Filex similar to those tested in this experiment series in order to minimise the risk of further resistance development until such time that alternative fungicides are approved.
- \* Where downy mildew occurs in crops, and where metalaxyl has been used, growers should consider submitting a sample for resistance testing.
- \* Growers should be aware of the opportunities for securing On or Off-Label Approval for one or more of the novel fungicides evaluated. Azoxystrobin (Amistar), whilst only providing moderate control of *Bremia* in this evaluation, is considered appropriate for further development in this crop primarily because of its activity on other important lettuce pathogens eg. *Botrytis*, *Sclerotinia*, *Phoma* and *Rhizoctonia*.

## **Practical and Financial Benefits from the Study**

The three-year study conducted during the period 1993 - 1996 has been of great practical significance. The work has demonstrated how serious infection by *B. lactucae* can be in situations where fungicides are either not applied or where metalaxyl is applied when the *Bremia* population is predominantly metalaxyl resistant. This has clearly demonstrated the importance of utilising appropriate Dm or R-genes in the lettuce cultivars and having available alternative fungicides with contrasting modes of action should new resistant strains occur.

During periods of high disease risk, particularly the autumn, when conditions favour the disease, growers are unlikely to be able to produce lettuce of sufficient quality without access to effective downy mildew fungicides. The loss of any authorised fungicide on a 'minor' crop can be serious and whilst the outcome of the EU dithiocarbamate review is not yet available, the potential loss of zineb caused considerable concern amongst the industry during the course of this study. Fortunately, the comparative study with mancozeb reported here has demonstrated equivalent efficacy against *B. lactucae* and therefore should zineb remain unsupported following the outcome of the EU review it is hoped that an alternative effective dithiocarbamate, ie. mancozeb, will remain available for use by lettuce growers. At the same time, previous opinion that dust formulations were more effective than WP formulations has been dispelled. It is unfortunate that lettuce propagators have lost the convenience of dust formulations but in view of the inferior efficacy it is difficult to justify pursuing a new UK approval.

The success of the integrated programmes evaluated has been of great practical significance. Even under severe disease pressure these programmes have effectively prevented downy mildew development and have significantly increased lettuce head weights. This clearly has important financial significance both in terms of improving the percentage of marketable heads in accelerating crop maturity.

None of the novel fungicides evaluated over the time course of this study were particularly outstanding against downy mildew. However, one product azoxystrobin (Amistar), has been singled out as a product potentially of immense value to the lettuce industry because of its activity against a wide range of pathogens which commonly occur in the crop. Assuming that either an On-Label or Off-Label can be secured this product should improve, and hopefully, simplify fungicide programmes in the lettuce crop. At the present time however this product does not have any approval for use on lettuce in the UK.

## EXPERIMENTAL SECTION

### Introduction

Downy mildew, caused by the oomycete fungus, *Bremia lactucae*, continues to be the most economically important disease in protected butterhead lettuce.

In the late 1970s chemicals, based on the phenylamide fungicide metalaxyl, provided an opportunity to maintain effective control using a relatively simple spray programme. Its use was widely adopted by the lettuce industry, in advance of UK approval, but unfortunately it was not long before resistant strains of the fungus appeared.

The occurrence of metalaxyl resistance in *Bremia* and the lack of effective alternative products, led to the development of complex strategy (McPherson & Crute, 1986) utilising Dm or R (resistance) genes in lettuce cultivars in combination with continued use of metalaxyl. This strategy has proved to be extremely valuable and, whilst requiring occasional modification as new metalaxyl resistant strains of *Bremia* have appeared, has provided an effective means of disease control for some 15 years now.

There is however a finite number of Dm or R genes in lettuce cultivars and it is probably only a matter of time before a new metalaxyl resistant strain of *Bremia* appears pathogenic on all commercial lettuce cultivars. It is important therefore that alternative fungicides are made available to the lettuce industry maintain effective disease control and to reduce the risk of further resistance development.

Pesticide approvals are under constant scrutiny and review procedures are now a routine component of the regulatory process. Dithiocarbamate fungicides are currently under EU review though the full outcome of this will not be available for some time. It is however considered unlikely that all dithiocarbamate products currently approved will be supported by the manufacturers. Whilst mancozeb, a partner mixture in most potato 'blight' fungicides is likely to be supported the single 'stand-alone' product zineb is likely to be under greater threat. Indeed, the dust formulation of zineb has already been lost. In anticipation of this possible withdrawal, alternative protectant fungicides need to be identified, as zineb has played a crucial role in mildew protection during lettuce propagation for many years.

During the course of this project several novel fungicides with activity against oomycete pathogens, including *Bremia*, have been launched either in the UK or elsewhere in Europe. Products with contrasting modes of action eg. strobilurins, are now available for use in the arable sector. They could be extremely useful in both maintaining effective disease control and in structuring an anti-resistance strategy on lettuce. It must be recognised however, that, in the UK, lettuce is only a 'minor' or specialist crop group and it is therefore unlikely that On-Label Approval for new products such as this will be developed specifically for the lettuce crop. Fortunately, to counter this withdrawal of support for minor 'horticultural' crops, a system of minor use authorisation has been put in place by the regulatory authority, PSD. Specific Off-Label Approvals (SOLAs) can now be secured once the necessary safety data, usually residues, has been generated. Therefore, once an effective product with good activity against *Bremia* has been identified it is now possible, assuming manufacturer support is not forthcoming, to take it forward (with grower approval) into the HDC sponsored SOLA programme to secure an approved use, assuming the data generated is satisfactory.

The aim of the present study has been to:

- a. Evaluate a range of novel fungicides with claimed activity against oomycete pathogens.
- b. Compare the efficacy of mancozeb with zineb.
- c. Evaluate dust formulations of mancozeb alongside a WP formulation.
- d. Compare their performance alongside the industry standard product, Favour 600SC and the widely promoted integrated programmes based around Aliette and Filex.

## **Materials and Methods**

### Site (1996)

(See also Annual Reports for 1993 and 1994.)

The trial reported here was located in a polytunnel at Horticulture Research International, Stockbridge House, Cawood, Selby, North Yorkshire, YO8 0TZ.

### Duration

A single trial was carried out during the high risk autumn period (July - November 1996).

### Cultivar

The cv. Titania, known to be susceptible to an introduced metalaxyl sensitive isolate of the downy mildew, was used in this trial.

### Trial Design

The trial was arranged in a Trojan Square design with four replicates of each treatment. Each plot consisted of five rows, each row containing 12 plants, with a total of 60 plants per plot. The outer plants in each plot acted as a picture-frame guard area. Plant spacing was 20 cm x 20 cm giving a plot size of 2.48 m<sup>2</sup>. The six outer rows on either side of the trial were retained as additional guard areas and, following artificial inoculation, provided high disease pressure on the treatment plots in the centre of the polytunnel. (For further details of trial layout see Annex I).



## Downy Mildew Inoculum

To evaluate the performance of fungicides either applied alone or in programmes it was necessary to ensure the presence of the disease during the whole of the trial period. To do this infected plants were placed in the crop. Seedlings of cv. Titania were inoculated with a strain of *B. lactucae* at Horticulture Research International, Wellesbourne. These infector plants were placed between trays of spare (guard) plants during the latter stages of propagation and once inoculum transfer had occurred the guard plants were planted in the two outer bays of six guard rows in the growing house (see trial plan in Annex I). The experimental (untreated and fungicide treated) seedlings were subsequently planted in the central two bays in the growing house.

## Treatments

1. Untreated control.

**Note: The unsprayed control treatment provides the base-line on which to assess the performance of the inoculated pathogen and the various fungicide treatments in the trial.**

2. Favour 600 SC (metalaxyl 100 g + thiram 500 g/l product) marketed by Novartis. Applied twice during propagation at 1-2 leaf stage (7 ml product/3 litres water/100 m<sup>2</sup> blocks) and 3-5 leaf stage (10 ml product/4 litres water/100 m<sup>2</sup> blocks) and twice within two weeks of planting out (dithiocarbamate restriction) at a rate of 15 ml product/5 litres water/100 m<sup>2</sup>.

**Note:** This product had previously provided effective control of downy mildew in lettuce. However, due to the development of fungicide resistance to the primary active ingredient, metalaxyl, its value in a fungicide programme is now more restricted. In a trial of this nature, using a metalaxyl sensitive isolate, it provides an important base-line from which to measure the performance of other fungicides or programmes.

3. Unicrop Mancozeb (80% WP) applied at a rate of 9.0 g product/100 m<sup>2</sup> blocks (half rate) during propagation at 3 day intervals followed by two applications, immediately after planting out and 14 days later, at 18 g product/100 m<sup>2</sup> lettuce.

**Note:** Dithiocarbamate products continue to be very effective protectant fungicides particularly when applied during propagation, where there are no restrictions on their use. Post-planting applications are now restricted up to two weeks post-planting (three weeks in the case of thiram on winter crops) because of the high risk of residues in the harvested crop, particularly in the slower growing winter months.

The outcome of the EU dithiocarbamate review will not be made available for some time though it is anticipated that additional data requirements will be requested for some of the ethylene-bis dithiocarbamates or EBDC's (zineb, mancozeb, maneb). Unfortunately the relatively high cost of generating the data is likely to mean that some active ingredients and some products will remain unsupported by the manufacturers. Products containing zineb are likely to be under most threat. As zineb has been an important and successful component in disease control programmes on lettuce for many years, alternative fungicides must be sought in case zineb remains unsupported. Mancozeb is used routinely as a partner mixture in most potato blight fungicides and is more likely to be supported. As mancozeb is also marketed as a 'straight' product and has an approved use on lettuce it is considered to be the main alternative dithiocarbamate to zineb and has therefore been included in this evaluation.

4. Mancozeb Dust (15%) applied every three days during propagation at a rate of 158 g product/100 m<sup>2</sup> blocks (half rate) followed by two applications post-planting at a rate of 316 g product/100 m<sup>2</sup> crop within two weeks of planting.

**Note: A Mancozeb dust formulation was made available by Ford-Smith as an experimental product only. (See also Note at 3. above).**

5. Integrated Programme 1 comprising:
  - a. Mancozeb (80% WP) applied at a rate of 9.0 g product/100 m<sup>2</sup> blocks (half rate) during propagation at three day intervals.
  - b. Filex (72% propamocarb hydrochloride) applied at the 2-3 leaf stage according to the SOLA document (No. 1625/95).
  - c. Favour 600 SC applied twice within 14 days of planting according to manufacturer's instructions.
  - d. Filex applied subsequently to Favour 600 SC at 14 day intervals (two sprays) according to SOLA (No. 1625/95) allowing 14 day harvest interval.

**Note: This integrated programme was selected to evaluate the performance of an intensive 'commercial' regime based around a protectant dithiocarbamate (wetable powder formulation of Mancozeb) in conjunction primarily with the Off-Label use of Filex.**

6. Integrated programme 2 comprising:
- a. Mancozeb dust (15%) applied at a rate of 158 g product/100 m<sup>2</sup> blocks (half rate) during propagation at three day intervals.
  - b. Aliette (80% w/w fosetyl-aluminium) marketed by Hortichem applied at the three leaf stage according to SOLA document (No. 0223/95), washing the product from the foliage immediately with clean water.
  - c. Favour 600 SC applied post-planting at the label recommended rate after four days.
  - d. Mancozeb (80% WP) applied 14 days post-planting (18 g product/100 m<sup>2</sup> lettuce).
  - e. Aliette applied at 21 days post-planting according to the SOLA document (No. 0223/95)
  - f. Filex applied subsequently at 14 day intervals (two sprays) according to SOLA (No. 1625/95).

**Note: This integrated programme was selected to evaluate the performance of an alternative intensive 'commercial' regime based around a protectant dithiocarbamate (dust formulation of mancozeb) in conjunction with the Off-Label use of Aliette primarily.**

7. Aliette (80% w/w fosetyl-aluminium) applied according to the SOLA (No. 0223/95) at the three leaf stage in propagation, and 7 and 21 days post-planting, washing the product from the foliage immediately after each application with clean water.

**Note: This treatment was included to a. evaluate the performance of Aliette applied alone according to the SOLA document, and b. to compare its performance when used in conjunction with a cellulose spray additive from Hortichem which was claimed to improve its performance. (See also Treatment 8. below).**

8. Aliette (80% w/w fosetyl-aluminium) applied according to the SOLA (No. 0223/95) at the three leaf stage in propagation 7 and 21 days post-planting in conjunction with a cellulose spray additive (M614 from Hortichem) at 1 litre product/100 litres water.

**Note: This treatment incorporating a cellulose spray additive was included to determine whether its use enhanced the performance of Aliette as claimed.**

9. Azoxystrobin (Amistar) marketed by Zeneca. Applied as a high volume spray to run-off two days post-planting and repeated at 14 day intervals at a rate of 0.8 litres product/1000 litres water.

**Note: This fungicide represents a totally new class of broad spectrum fungicides, the strobilurins, originally derived from a toadstool. Azoxystrobin has claimed activity against downy mildew, powdery mildew, *Rhizoctonia*, *Botrytis cinerea* and various leaf-spot fungi eg. *Phoma*, *Septoria*. If these claims can be substantiated then strobilurins could potentially revolutionise approaches to disease control in many horticultural crops including lettuce. Due to a moderate risk of fungicide resistance developing in pathogen populations, product stewardship will be important. The manufacturers are likely to apply restrictions on its use pattern to minimise the resistance risk. It is vitally important that mis-use does not occur.**

10. CGA 245704 ('Bion') marketed by Novartis. Applied as a high volume spray to run-off two days post-planting and repeated at 14 day intervals at a rate of 2.5 g product/100 l water. Maximum of four sprays with a 14 day harvest interval.

11. CGA 245704 ('Bion') Applied as a high volume spray to run-off two days post-planting and repeated at 14 day intervals at a rate of 5.0 g product/100 l water. Maximum of four sprays with a 14 day harvest interval.

**Note: Two rates of a new 'Plant Activator' from Novartis were included to evaluate its claimed performance against *Bremia* in this experiment. 'Bion' is the first of a new generation of plant protection products which function by triggering defence systems in the host. Its extremely low use pattern and excellent safety data package merits its inclusion here.**

12. Invader (7.5% dimethomorph + 66.7% mancozeb) marketed by Cyanamid. Applied as a high volume foliar spray at a rate of 7.5 g product/2.56 l water and 10 g product/5.0 l water/100 m<sup>2</sup> blocks during propagation at 100% emergence and at 2-3 leaf stage and twice within two weeks of planting out at a rate of 15 g product/17.5 l water/100 m<sup>2</sup> crop.

**Note: The performance of Invader was a little disappointing in the 1994 experiment yet subsequent reports on its efficacy against related oomycete pathogens eg potato blight, merited a further evaluation against *Bremia* in 1996.**

### Fungicide Application

Fungicide applications in propagation were made using a hand held 'Polyspray' sprayer. The dust treatments in propagation and post-planting were applied by pepper pot shakers. HV spray application post-planting were made using an Oxford Precision Knapsack spray (E-Bar Engineering) with boom attachment modified to operate with compressed air.

### Crop Husbandry

The crop was grown to a good commercial standard and was maintained in a healthy state (with the exception of the introduced pathogen) according to normal horticultural practice and produced adequate yields for representative purposes. Pesticides were used occasionally, as necessary, but were chosen so as not to affect the aim of the investigation.

### Crop Diary and Cultural Details

Seed of the cv. Titania were sown into 3.8 cm peat modules on 13 August, covered and placed in a cold store at 1-2°C for 24 hours to aid germination. The seeds were moved to a propagation unit (F20) on 14 August. On 19 August a germination assessment was conducted when emergence was complete.

On 3 September the spare 'guard' plants were transferred to a separate polythene tunnel and 'infecter' plants with a virulent, but metalaxyl sensitive, strain of *B. lactucae* were introduced and subsequently covered with polythene to aid infection and establishment.

Basilex was applied to the soil in the trial area on 4 September prior to planting for the control of bottom-rot (*Rhizoctonia solani*) and the trial was planted out on 5 September. The inoculated guard plants down either side of the trial area were planted on 6 September. Full details of fungicide spray timings are provided in Table 1.

Interim assessments of downy mildew were conducted on 24 September and 2 October. The trial was harvested on 23 October when a final disease assessment was carried out and untrimmed and trimmed yield measurements were made.

## Disease Assessments

During crop development interim assessments of downy mildew were made visually from above the crop to estimate the percentage leaf area infected with *B. lactucae*. From this data a mean % leaf area per treatment was calculated.

In addition, interim assessments of plant vigour were made on whole plots using the following 0-3 scale where:

- 0 = Extremely poor vigour, plants stunted.
- 1 = Growth weak, plants not thriving.
- 2 = Growth moderate, but smaller than the best plots.
- 3 = Plants excellent, thriving.

At harvest on 23 October, detailed assessments of downy mildew were made when 20 individual plants were cut to allow closer examination of the lower leaves. On each plant the infection severity was assessed using a 0-3 scale where:

- 0 = No visible infection.
- 1 = Slight infection on lowest leaves causing slight yellowing. No additional trimming required, plant marketable.
- 2 = Moderate leaf infection with slight spread to younger leaves. Additional trimming required, resulting in a marketable plant but with a reduced head weight.
- 3 = Severe leaf infection with extensive infection on younger leaves. Excessive trimming required to remove affected leaves resulting in an unmarketable plant.



From this data a disease index was calculated as follows:

$$\frac{1 (\text{No in category 1}) + 2 (\text{No in 2}) + 3 (\text{No in 3}) \times 100}{\text{No of plants assessed} \quad 3}$$

The range of the index was, therefore, 0 (no disease) to 100 (most severe disease).

In addition, the percentage leaf area infected by *B. lactucae* was assessed visually on each of the 20 heads harvested.

### Yield Determination

At harvest 20 plants/plot were cut from each plot and bulk weighed to give a mean untrimmed lettuce head weight. Each lettuce was then trimmed to a commercial standard, removing any infected leaf tissue from the base to provide a clean frame. The lettuce were then re-weighed to provide a mean trimmed lettuce head weight.

The difference between the untrimmed and trimmed yield was therefore regarded as the weight loss as a result of leaf infection by downy mildew.

### Statistical Analysis

The data from this trial was subjected to an analysis of variance and significance determined at a 5% level of probability ( $P = 0.05$ ). Significance is indicated by:

- \* Significant at the 95% level of probability.
- \*\* Significant at the 99% level of probability.
- \*\*\* Significant at the 99.9% level of probability.

Differences between treatments can be compared (at the 95% probability level) using the Least Significant Difference (LSD) figure at the bottom of each column. It should be noted that significance tests are only approximate as the data is not fully normally distributed.

#### Storage of Data

The raw data from this trial will be stored for a period of not less than 5 years in the HRI archive at Stockbridge House. Access to the data can only be made via the designated archivist.

#### Official Recognition and Quality Assurance

This trial was conducted in accordance with the draft guidelines for official recognition of efficacy testing organisations as outlined by the UK pesticide regulatory authority, PSD. See ref PRD 2400/2995 for more information.

A specific quality assurance audit was not undertaken in the trial.

## Results

(See also Annual Report for 1993 and 1994).

Following artificial introduction of *B. lactucae* in this trial the disease was relatively slow to develop. A change to cooler, wet weather aided disease establishment though it was judged that isolates of *Bremia* other than that introduced predominated. The introduced isolate was metalaxyl sensitive and therefore effective control could have reasonably been expected from applications of Favour 600 SC. Yet, the performance of this product deteriorated considerably during the time course of the experiment and at harvest the head weight was poorer than that of the untreated.

In an interim assessment on 24 September (Table 2) crop vigour remained fairly high in most treatments and the % leaf area with *B. lactucae* was relatively low. The vigour was reduced significantly in the untreated control compared to most other treatments and this treatment had the highest leaf area infection of 12.5% at this early stage. Already some treatment differences were becoming apparent and the 'Plant Activator' (Bion) treated plots were surprisingly poor.

Subsequently, the disease progressed rapidly and by 2 October (Table 3) the vigour in the untreated control had declined dramatically and the % leaf area infection had risen to 43.8. Favour 600 SC continued to perform reasonably well at this point in the experiment though a leaf area infection of 11.3% suggested that metalaxyl-insensitive strains were already present in the population.

The dithiocarbamate mancozeb applied as a WP formulation provided excellent disease suppression at this mid-point in the trial and provided significantly better control of downy mildew than the dust formulation of mancozeb.

Both integrated programmes and the Aliette treatments (+/- cellulose spray additive) maintained excellent disease control by 2 October and it was extremely difficult to find mildew infection on the leaf tissues at this stage.

The new strobilurin fungicide Amistar was a little disappointing as a moderate infection level could be found in treated plots. However, compared to the untreated and Bion treated plots it was providing a good suppression of the disease. Plants treated with Bion had extremely high disease levels and there was no indication of disease control at this stage. Invader also provided only moderate control at this stage in the experiment and was equivalent to Amistar, though more sprays had been applied.

At harvest on 23 October the disease level in the unsprayed control was extremely high and this provided an exceptionally stern test for the fungicides evaluated.

The performance of Favour 600 SC declined considerably and by crop maturity provided only a marginal improvement compared to the untreated (Table 4). Interestingly, the untrimmed and trimmed lettuce head weights were marginally, though not significantly, lower than the untreated control (Table 5).

Mancozeb WP, applied as a protectant product with a restricted use pattern post-planting performed extremely well though unfortunately was let down by poor persistence in the later stages of the experiment. Perhaps surprisingly, Mancozeb dust by comparison was slightly poorer in interim assessments (Table 3) and produced a significantly lower untrimmed head weight at harvest (Table 5).

The two integrated programmes provided exceptional mildew control, particularly given the high disease pressure. Both programmes significantly reduced the disease and increased the lettuce head weight compared to the untreated control. Interestingly, comparing the two integrated programmes, the latter programme (2) was superior to the former programme (1) and both the untrimmed and trimmed head weights were increased considerably as a result (Table 5). A yield increase of 106% was recorded for the trimmed lettuce in integrated programme 2 compared to the untreated control.

The Aliette SOLA treatment applied alone also gave outstanding control though the addition of the cellulose spray additive did not improve its performance.

Treatment with Amistar provided a significant reduction in lettuce downy mildew applied as a stand-alone product though was over-shadowed by the level of disease control achieved with the integrated programmes. Amistar did provide a significant increase in the untrimmed lettuce head weight though much of this gain was lost in trimming.

The novel 'Plant Activator' was extremely disappointing in this experiment and no evidence for disease control was secured. Indeed there was even some indication that treatment exacerbated downy mildew in this evaluation.

Finally, treatment with Invader, whilst providing some suppression of *Bremia* was clearly not able to cope under these high disease pressure conditions and no increase in lettuce head weight was recorded.

**Table 2: Interim plot assessments for plant vigour and downy mildew on 24 September 1996.**

Treatment	Crop Vigour Index* (0-100)	% Leaf Area with* Downy Mildew
Untreated	75.0	12.5
Favour 600 SC	100.0	2.0
Mancozeb 80% WP	91.7	0.0
Mancozeb 15% Dust	100.0	1.5
Integrated Programme 1	100.0	0.3
Integrated Programme 2	100.0	0.0
Aliette (SOLA)	100.0	0.0
Aliette (SOLA) + Adjuvant	100.0	0.0
Amistar	91.7	3.0
Bion (2.5 g)	91.7	8.3
Bion (5.0 g)	83.3	9.0
Invader	100.0	1.8
Significance	*	***
SED (30 df)	7.03	1.16
LSD (5%)	14.35	2.37

\* Assessments based on visual examination of whole plots from above.

**Table 3: Interim plot assessments for plant vigour and downy mildew on 2 October 1996.**

Treatment	Crop Vigour Index* (0-100)	% Leaf Area with* Downy Mildew
Untreated	33.3	43.8
Favour 600 SC	83.3	11.3
Mancozeb 80% WP	91.7	9.5
Mancozeb 15% Dust	66.7	25.0
Integrated Programme 1	100.0	0.5
Integrated Programme 2	100.0	0.0
Aliette (SOLA)	100.0	0.0
Aliette (SOLA) + Adjuvant	100.0	0.0
Amistar	75.0	21.3
Bion (2.5 g)	41.7	46.3
Bion (5.0 g)	41.7	43.8
Invader	75.0	14.3
Significance	***	***
SED (30 df)	8.19	4.183
LSD (5%)	16.73	8.542

\* Assessments based on visual examination of whole plots from above.

**Table 4: Final assessments of crop vigour and lettuce downy mildew infection at harvest on 23 October 1996.**

Treatment	Crop Vigour Index (0-100)	% Leaf Area with* Downy Mildew	Downy Mildew* Index (0-100)
Untreated	33.3	23.8	91.0
Favour 600 SC	58.3	33.8	86.7
Mancozeb 80% WP	75.0	38.8	33.3
Mancozeb 15% Dust	66.7	27.5	66.0
Integrated Programme 1	100.0	10.0	11.7
Integrated Programme 2	100.0	0.8	0.7
Aliette (SOLA)	100.0	3.3	19.3
Aliette (SOLA) + Adjuvant	100.0	11.3	32.0
Amistar	91.7	11.3	28.7
Bion (2.5 g)	41.7	27.5	88.3
Bion (5.0 g)	41.7	40.0	91.7
Invader	66.7	35.0	41.7
Significance	***	***	***
SED (30 df)	7.61	3.24	6.97
LSD (5%)	15.53	6.8	14.24

\* Assessments based on assessment of 20 individually harvested lettuce per plot.



**Table 5: Untrimmed and trimmed head weight of lettuce at harvest on 23 October 1996.**

Treatment	Mean Untrimmed* Head Weight/Plant (g)	Mean Trimmed* Head Weight/Plant (g)
Untreated	162.5	122.5
Favour 600 SC	148.0	106.4
Mancozeb 80% WP	202.0	118.1
Mancozeb 15% Dust	164.5	119.0
Integrated Programme 1	228.0	163.9
Integrated Programme 2	266.0	251.4
Aliette (SOLA)	232.0	195.3
Aliette (SOLA) + Adjuvant	242.0	182.3
Amistar	204.5	137.5
Bion (2.5 g)	146.0	97.0
Bion (5.0 g)	148.5	109.8
Invader	188.5	129.3
Significance	***	***
SED (30 df)	14.27	17.56
LSD (5%)	29.14	35.86

\* Mean lettuce head weight calculated from harvesting and weighing 20 individual lettuce heads in each of 4 replicate plots.

## Discussion

(See also Annual Report for 1993 and 1994).

The apparent natural occurrence of metalaxyl-insensitive strains in the *Bremia* population has clearly highlighted the danger of relying on metalaxyl as Favour 600 SC for the control of downy mildew, without the necessary consideration of the Dm or R-gene resistance in the cultivar. It is evident that where effective Dm or R-genes are not available, alternative strategies to Favour 600 SC must be made available if effective control is to be maintained.

The Off-Label use of Aliette and Filex, secured in previous HDC funded studies, has clearly been worthwhile as evidence by their effectiveness in the integrated programmes deployed in the experiment reported. Unfortunately, the occasional reports of crop damage following application of Aliette ensure there is a reluctance within the industry to use the product routinely. Yet, symptoms of phytotoxicity have not been reported during the course of this work. From the evidence provided here Aliette would appear to be superior to Filex against *Bremia*. However, due to the restricted use of Aliette during the later stages of the crop (latest application 21 days post-planting), the use of Filex with a 14 day harvest interval, remains extremely important as it is the only product which can be applied towards crop maturity particularly during the long Autumn and Winter periods.

From the results obtained here there would still appear to be considerable merit in maintaining a full dithiocarbamate programme during propagation and in the first two weeks post-planting. Mancozeb 80% WP appears to be most effective and approved products are available for use on lettuce. The relative poor performance of the dust formulation indicates it is not worth pursuing, from an efficacy standpoint, even though it is recognised that there is a grower preference for dust formulations during propagation. It will be difficult therefore to justify the cost of pursuing a new approval on the grounds of convenience particularly when there is already an approved use of a more efficacious product.

Amistar, the first of a new generation of strobilurin fungicides, was a little disappointing against downy mildew in this trial. It should be noted however that disease pressure was much higher than that normally experienced in commercial practice. Because of the broad spectrum nature of the product, particularly against the primary diseases affecting lettuce eg. *Botrytis*, *Sclerotinia*, *Rhizoctonia*, *Bremia* and *Phoma* it is recommended that either an On or Off-Label Approval for its use is pursued as soon as possible. In this respect discussions are in progress with the manufacturers with a view to securing a full data package for the use of Amistar in a range of horticultural crops, including lettuce.

Finally, the extremely poor result with Bion, the novel 'Plant Activator' from Novartis was particularly surprising and this needs to be further investigated before further studies are undertaken with the product.

## Conclusions

- \* In this experiment metalaxyl (as Favour 600 SC) could not be relied upon to provide effective control of *B. lactucae*, even though the isolate originally introduced was metalaxyl sensitive, and this was considered to be due to the natural introduction of metalaxyl resistant strains of *B. cinerea*.
- \* The dithiocarbamate fungicide, mancozeb, provided excellent control of downy mildew in the early stages of the trial. Its restricted use pattern post-planting prevented control being maintained and its efficacy deteriorated through to crop maturity.
- \* Mancozeb 80% WP was superior to Mancozeb 15% Dust in this evaluation. This is contrary to popular commercial opinion, where zineb dust has been used previously. The preference towards dust formulations may relate more to the convenience of the application procedure by lettuce propagators and growers rather than on the overall merits on efficacy. Yet, the argument that wet sprays of WP formulations exacerbate downy mildew do not appear to stand. It is therefore, difficult to justify a case for pursuing a new approved use of Mancozeb Dust to replace Zineb dust when Mancozeb WP is so effective.
- \* The two integrated programmes were outstanding in this evaluation, particularly considering the high disease pressure in the trial area. Both Aliette and Filex, as important components in the programmes have individual merit. Aliette was more efficacious though is restricted in its use pattern to 7 and 21 days post-planting. Filex whilst slightly less effective can be used much later, nearer crop maturity providing a 2 week harvest interval is maintained. Growers should be encouraged to formulate and use the same or similar integrated programmes until such time that alternative effective products become available.

- \* Of the novel fungicides evaluated (Amistar, Bion and Invader) none provided control of downy mildew equivalent to the integrated programme. However, Amistar has been singled out for particular attention not because of its moderate suppression of *Bremia* but because, as a novel protectant broad spectrum fungicide, it is likely to reduce but not eliminate, the need for complex spray programmes in the future. It is therefore recommended that data is generated as soon as possible to secure either an On-Label or Off-Label for the use of Amistar on lettuce. Because of its broad spectrum activity it is likely to be effective in reducing the number of sprays applied for *Botrytis*, *Rhizoctonia*, *Sclerotinia* and leaf-spot fungi.

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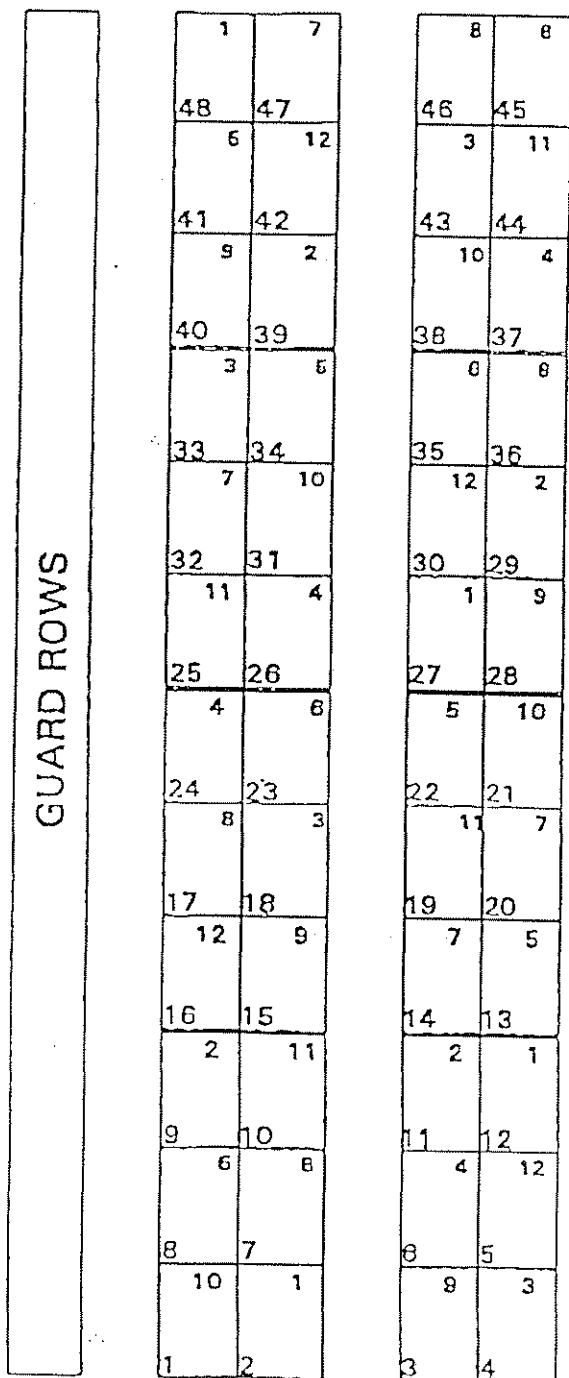
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**Annex 1: Trial plan for the replicated experiment on downy mildew in lettuce conducted in 1996.**

Protected Lettuce: Fungicide Control Of Downy Mildew. (1996)  
 North Polytunnel HRI/SH/PP/96/369



← North

Treatments

- T1 Untreated control
- T2 Favour 60SC
- T3 Mancozeb 80%WP
- T4 Mancozeb 15% dust
- T5 Integrated programme 1
- T6 Integrated programme 2
- T7 Aliette (SOLA)
- T8 Aliette (SOLA) + adjuvant
- T9 Amistar
- T10 Bion (CGA245704 – low rate)
- T11 Bion (CGA245704 – high rate)
- T12 Invader

Plot area : 2.48sq.m. 5 x 12 plants (60/plot)