Project title:	Protected Lettuce: Developing best practice for disease control
Project number:	PC 298
Project leader:	Dr G M McPherson
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Date project completed (or expected completion date):	December 2010

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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CONTENTS

GROWER SUMMARY

Headline

- A series of spray programmes, based on 2 or 3 application timings provided good control of key lettuce pathogens.
- Basilex used at half the manufacturers label rate provided effective control of bottom-rot caused by *Rhizoctonia solani* when used as part of a spray programme.
- Fubol Gold used at a reduced rate appeared to provide useful control of downy mildew when used as part of a spray programme.

Background

There have been several recent approvals of new fungicides for use on protected lettuce e.g. Amistar (azoxystrobin), Signum (boscalid + pyraclostrobin), Scala (pyrimethanil), Teldor (fenhexamid), Switch (cyprodinil + fludioxonil) and the biological control agents Contans (*Coniothyrium minitans*) and Serenade (*Bacillus subtilis*). These products provide a useful addition to the range of tools available to help control foliar pathogens in the crop, and may, in the future, be supplemented by other products currently in the approval pipeline e.g. Revus (mandipropamid). However, information from growers suggests that there is a lack of clarity and confidence about the most efficient spray programmes to achieve effective disease control. This initial study investigated both the use and single products and the use of different combinations of products in spray programmes designed to enable comparisons of their efficacy against a range of different lettuce pathogens.

The information gathered from this work is aimed at providing growers with a greater understanding of the range of disease management options currently available to assist in the delivery of effective use programmes. An important part of this work was to develop the use of less hazardous crop protection products and also to switch to low residue risk products towards the end of the spray programmes to minimise the likelihood of maximum residue level (MRL) exceedences following routine residue testing.

It is envisaged that this study will initiate a rolling-programme of crop protection product evaluation in protected lettuce to ensure new actives and biocontrol measures are evaluated for effective incorporation into integrated programmes. Within the short time-frame of this initial study three primary pathogens of lettuce were studied; *Sclerotinia sclerotiorum*, downy mildew and *Rhizoctonia solani.* Additional data were also collected on other naturally occurring pathogens e.g. *Botrytis cinerea* during the study.

Summary

Three fully replicated fungicide evaluation experiments were carried out between autumn 2009 and autumn 2010 on protected lettuce. Each experiment was carried out in the same 150m^2 glasshouse using a susceptible cultivar of butterhead lettuce. The aim was to establish a 'disease nursery' following inoculation with the appropriate pathogens. Each experiment was inoculated with the respective pathogen in an effort to provide sufficient challenge for the products. A series of products was selected against the introduced pathogen for use individually and as part of a series of spray programmes.

Experiment 1 (autumn 2009) focused on the control of *Sclerotinia sclerotiorum.* A range of fungicides including Amistar (azoxystrobin), Teldor (fenhexamid), Signum (boscalid + pyraclostrobin), Switch (cyprodinil + fludioxonil), Rovral (iprodione), Scala (pyrimethanil) and Contans (*Coniothyrium minitans*) were used as individual products (albeit repeatedly) or as components within spray programmes.

Low levels of *Sclerotinia* developed in this crop therefore little data was gathered. Residue testing was carried out on the treated crop and no MRL exceedences were reported.

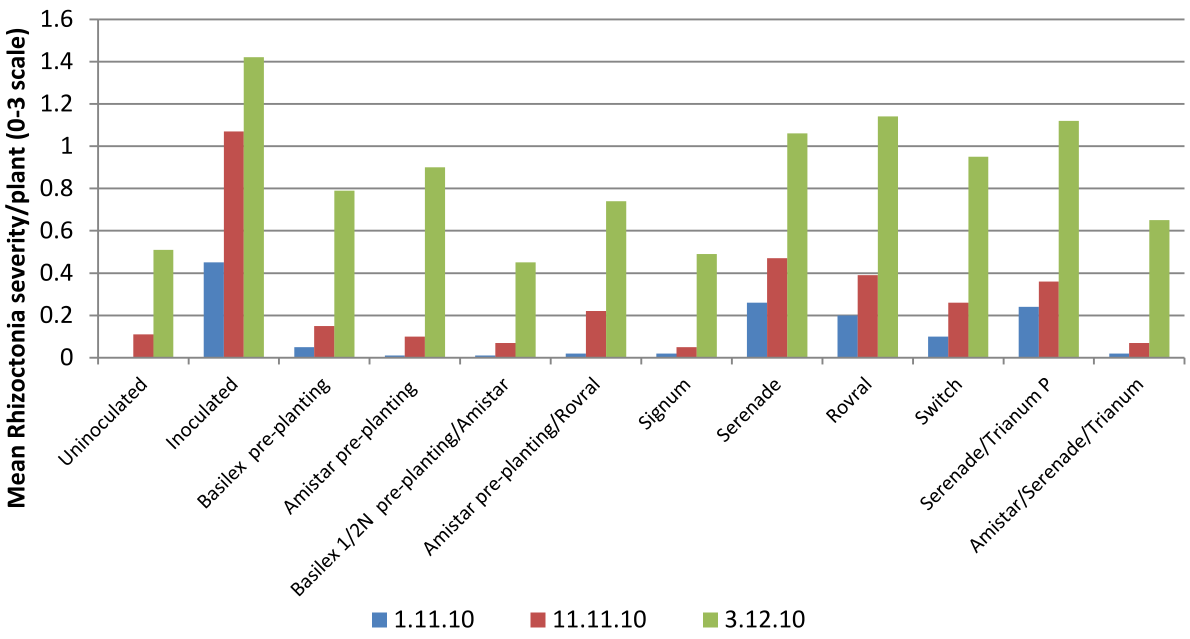
Trial 2 in spring 2010 studied fungicide effects on downy mildew study. The cultivar 'Cobham Green' was used, which contains very few *Bremia lactucae* (BL) resistance genes and therefore is highly susceptible. The seed and inoculum for this study were supplied by the research institute Naktuinbouw in Holland. Fungicide treatments in this study included Amistar, Fubol Gold (mancozeb + metalaxyl-M), Signum, Switch, Revus (mandipropamid), Previcur Energy (fosetyl-aluminium + propamocarb hydrochloride) together with Valbon (benthiavalicarb + mancozeb) and DP98 (phosphonate).

Downy mildew (DM) infection established well in the guard rows between the plots following inoculation. However, a spell of unseasonably warm weather halted progress of the infection and prevented rapid movement into the experimental area so the disease only developed at low levels. Data gathered on the control of downy mildew suggested that Revus, Previcur Energy and two of the experimental programmes where the same products were used in half-rate tank mixes with Fubol Gold appeared to be effective. The researchers also gathered information on the incidence and severity of *Sclerotinia* in this crop as a moderate naturally-occurring infection was observed. The inclusion of the strobilurin fungicides Amistar and Signum provided significant control of this pathogen.

No exceedences of MRLs were recorded on the sampled lettuce from this study.

The third study, carried out in autumn 2010, primarily investigated the efficacy of products against *Rhizoctonia* bottom-rot. Basilex (tolclofos-methyl) and Amistar as pre-planting treatments were compared with post-planting applications of Amistar, Rovral, Signum, Serenade and Trianum P (*Trichoderma* spp.) to control the disease. The results of this trial are given in the chart below:

Severity of *Rhizoctonia* bottom-rot following treatment application over 3 assessment dates during the study



The standard pre-planting treatments were carried out in treatments 3-6, providing a good level of control, particularly in the early stages of the study. Treatment 5 used Basilex applied at half normal label rate and followed up 2 weeks later with a single application of Amistar. Treatment 7, 2 post-planting applications of Signum, also provided a good level of control. Full details of the treatment programmes and results are provided in the Science section of the full report.

None of the harvested lettuce exceeded the MRL in this study

Financial Benefits

These initial studies, whilst not at a stage where a robust cost-benefit analysis can be conducted, do provide an indication that effective spray programmes can be devised which contain one or two treatments, although this does depend on the overall incidence and severity and disease. Also, some products are effective when used at lower than the manufacturer's recommended rate when disease pressure is not too severe. This approach needs further evaluation and consideration with respect to the potential development of resistance in the fungal organism, but is widely accepted as common practice in the arable sector.

The studies conducted also involved substitution of lower risk products such as biological control products e.g. Serenade and Trianum P to reduce the possibility of pesticide residues on crops. This strategy may well provide a good option for growers in the future if adequate levels of disease control can be demonstrated.

Action Points

- Consider products for inclusion in spray programmes based on their full spectrum of activity rather than the label advice only.
- Consider pre-planting treatments where appropriate to reduce the number of products required for disease control post-planting.
- Consider the application of products at lower than the full label rate where disease pressure is low and where reduced-rate efficacy has been demonstrated.
- Consider substituting biological control products or alternative non-chemical products for fungicides as crops attain maturity to reduce risk of MRL exceedences where efficacy can be demonstrated.

SCIENCE SECTION

Introduction

Over recent years there have been a number of approvals of new pesticides for the protected lettuce industry e.g. Amistar (azoxystrobin), Signum (pyraclostrobin & boscalid), Scala (pyrimethanil), Teldor (fenhexamid), Switch (cyprodinil & fludioxonil) and the biological control agents Contans (*Coniothyrium minitans*) and Serenade (*Bacillus subtilis*). Although these new products are welcomed and integrated by lettuce growers there is a lack of confidence in how best to use these products either alone or in combination to control many of the common soil and air-borne pathogens of lettuce. This initial short-term study focused on using the products individually or in integrated spray programmes to investigate and demonstrate their efficacy to control three of the main diseases of lettuce, notably *Sclerotinia sclerotiorum*, Downy mildew (*Bremia lactucae*) and bottom-rot (*Rhizoctonia solani)*.

A 150m^2 dedicated glasshouse was used at STC to establish a 'disease nursery'. Using this site three protected lettuce crops were grown at STC between September 2009 and December 2010 to enable efficacy work on each of the stated pathogens to be carried out at a suitable time of year when the most favourable environmental conditions for pathogen development could be expected. Whilst the crops were inoculated with their respective pathogens; they were also assessed for a range of common lettuce pathogens to gather efficacy data on other (non-targeted) organisms e.g. *Botrytis*, *Phoma* etc. Residue data was collected at the end of each study to ensure that there were no exceedences of MRLs.

Materials and methods

Trial Design

Each of the three trials was undertaken in the same 150m^2 glasshouse at STC. They incorporated 12 treatment regimes with 4 replicate plots laid out in a randomized block arrangement. Randomisations were generated using Agricultural Research Manager (ARM) software (trial plans in Appendix 1). Spray applications were made using an Oxford Precision knapsack sprayer and a 3 nozzle boom. Sprays were applied at a standardized 2 bar pressure.

Figure 1. General view of trial area.

Trial 1. Primary Target: *Sclerotinia sclerotiorum*

Timing: Autumn of 2009.

Crop: Butterhead lettuce cv. Wiske

Location: Fairfield 6 at STC.

Treatments

Table 1. Details of products and spray programmes used in Trial 1.

Trt No.	Product	Active Ingredient	Application rate/ha	Application Timing				
				Pre-plant	1-2 days post-planting	7-10 days post-planting	14-21 days post-planting	28-35 days post-planting
1	Untreated	-	-	-	-	-	-	-
2	Signum (Si)	pyraclostrobin + boscalid	1.5 kg	-	+	-	+	Optional
3	Amistar (A)	azoxystrobin	0.5 l (2 x ½ rate)	-	+	-	+	Optional
4	Switch (Sw)	cyprodinil + fludioxonil	0.6 kg*	-	+	-	+	Optional
5	Teldor (T)	fenhexamid 50% w/w	1.5 kg	-	+	-	+	Optional
6	Rovral WG	iprodione	33 g/100 l	-	+	-	+	Optional
7	Serenade	*Bacillus subtilis*	10 l	-	+	-	+	Optional
8	Scala	pyrimethanil 400g/l	1.32 l	-	+	-	+	Optional
9	Contans (C)	*Coniothyrium minitans*	4 kg	+	-	-	-	-
10	C+Si+A+Sw+T	as above for each component	as above for each component	+ C	+Si	+A	+Sw	+T
11	C+A+Si	as above for each component	Amistar at 1 l others as above	+C	+A	-	+Si	-
12	C+Si+Sw	As above for each component	As above for each component	+C	-	+Si	-	+Sw

* Used at incorrect higher rate of 1.5 kg for 1st application

Diary

24.09.09	Trial area inoculated with vernalised sclerotia
24.09.09	Pre-planting treatments (Contans) applied
30.09.09	Crop planted
2.10.09	1st spray applied (2 days post-planting)
8.10.09	2nd spray applied (8 days post-planting)
15.10.09	3rd spray applied (15 days post-planting)
20.10.09	Full disease assessment carried out
3.11.09	Full disease assessment carried out
16.11.09	Final spray application (46 days post-planting)
1.12.09	Final assessment and harvest; samples collected for residue analysis.

Inoculation with *S. sclerotiorum*

Sclerotia previously collected from infected lettuce crops and stored in a refrigerator (4°C ±2°) were used to inoculate the trial area. Viability was checked by plating out sclerotia onto artificial growth media (potato dextrose agar) prior to inoculation. Approximately 50 sclerotia per plot were pushed into the top few centimeters of soil between the planting points of the marked-out plots prior to the application of the Contans (Treatments 9-12). The trial area was kept moist for the week prior to planting.

Disease Assessments

The crop was assessed on 3 occasions. Data was collected on 10 plants per plot. During the final disease assessment 10 heads per plot were cut at soil level and inverted for assessment. The heads were then weighed, trimmed and re-weighed to provide data on the weight of lettuce lost due to trimming away of infected material.

Disease assessment scales

S. sclerotiorum only

0 – No disease present

1 – Slight discoloration present on basal area of plant

2 – Moderate infection – mycelium present and plant starting to collapse

3 – Severe infection – plant collapsed, mycelium and/or sclerotia present

General disease incidence

0 – No disease

1 – Slight infection affecting bottom leaves only

2 – Moderate infection affecting bottom leaves and moving up head

3 – Severe infection >50% of plant affected.

Residue Data

Lettuces from each treatment were retained (frozen) for residue testing.

Trial 2. Primary Target: *Bremia lactucae*

Timing: April – June 2010
Crop: Butterhead lettuce cv. Cobham Green – chosen for its lack of downy mildew resistance genes.
Location: Fairfield 6 at STC.

Treatments

Table 2. Details of treatments and application timings for trial 2.

Treatment	Active ingredient(s)	2-3 days post-planting	7-14 days post-planting	17-21 days post-planting	7 days pre-harvest (optional) †
1 Untreated control	Water only	+	+	+	+
2 Standard programme	see products	Amistar	Fubol Gold	Signum	Serenade
3 Revus*		Revus	Revus	Revus	Revus
4 Signum *		Signum	Signum	Signum	Signum
5 Serenade *		Serenade	Serenade	Serenade	Serenade
6 Previcur Energy*		PE	PE	PE	PE
7 Exp. Prog. 1	Various	FG/Revus tank mix (half rate)	-	FG/Revus tank mix (half rate)	-
8 Exp. Prog. 2	See products	Valbon	Signum	DP98	DP98
9 Exp. Prog. 3	Various as listed earlier	Revus	FG	PE	Serenade
10 Exp. Prog. 4	Various as listed earlier	Amistar/FG tank mix (half rate)	PE/Switch tank mix (half rate)	-	Serenade
11 Exp Prog. 5	Various as listed earlier	FG/Revus tank mix (half rate)	Serenade	Signum/Switch tank mix (half rate)	Serenade
12 Exp. Prog. 6	Various as listed earlier	PE/Signum tank mix (half rate)	FG/Switch tank mix (half rate)	Serenade	Serenade

* Treatments are experimental so HI can be ignored for the purposes of this trial. Residue data not required.
† This optional treatment not applied.

Table 3. Application rates used in trial 2

Product	Active	a.i. concentration	Application rate/ha
Amistar	azoxystrobin	250g/l	1 l
Fubol Gold	metalaxyl-M + mancozeb	64:4% w/w	1.25 kg†
Signum	boscalid + pyraclostrobin	26.7:6.7% w/w	1.5 kg
Serenade	*Bacillus subtilis*		10 l
Revus	mandipropamid	250g/l	0.6 l
Previcur Energy	fosetyl-Al + propamocarb-HCl	310:530 g/l	2.5 l*
Switch	cyprodinil + fludioxonil	37.5:25 w/w	0.6 kg
Valbon	benthiavalicarb isopropyl + mancozeb	1.75:70 w/w	1.8 kg
DP98	phosphonates		4 l

† Used at lower than recommended field rate * Used at incorrect rate in 1st application .

Crop Diary

22.04.10	Trial planted
30.4.10	DM inoculum applied to guard plants between plots
5.05.10	1st spray application
17.5.10	Full disease assessment carried out
17.5.10	2nd spray application
26.5.10	3rd spray application
7.06.10	Full disease assessment and harvest

Inoculation with *Bremia lactucae*

Batches of Cobham Green seedlings pre-infected with *Bremia* were supplied by the Dutch research institute Naktuinbouw for this study. On receipt the spores were washed from the seedlings using sterile distilled water to produce a spore suspension. The spores were sprayed onto the pre-wetted guard plants between the plots of lettuce using a small hand sprayer at the end of the day. The inoculated lettuce was covered with clear polythene overnight to maintain leaf wetness and thereby provide optimum conditions for infection.

Disease Assessments

The crop was assessed on 2 separate occasions. During the first assessment 10 plants per plot were scored for the incidence and severity of downy mildew and also for *Sclerotinia* as plants showing symptoms of this basal rot pathogen were noted. The final disease assessment was carried out prior to harvesting the crop. On this occasion 20 plants per plot were assessed and untrimmed and trimmed yield was recorded. Severe infection with *Sclerotinia* had had an impact on the number of lettuce heads per plot which were suitable for trimming. Where possible a minimum of 10 heads were weighed, trimmed and then re-weighed, but where this was not possible the actual number of heads harvested was recorded and assessment data adjusted accordingly.

The disease scales detailed in the Trial 1 methods section were employed for these assessments.

Residue Data

Lettuce from each treatment were retained (frozen) for residue testing.

Trial 3. Primary Target: *Rhizoctonia solani*

Timing: October – December 2010
Crop: Butterhead lettuce cv. Trinette
Location: Fairfield 6 at STC*.

Treatments

Table 4. Details of treatments and application timings for Trial 3

Treatment No.	Application timing			
	Pre-planting	**2-3 days post-planting**	**7-14 days post-planting**	**14-28 days post-planting**
1 (uninoc control)	-	-	-	-
2 (inoc control)	-	-	-	-
3	Basilex - full	-	-	-
4	Amistar	-	-	-
5	Basilex - half	-	Amistar	-
6	Amistar	-	Rovral WG @ 14 day intervals	
7	-	Signum*	-	Signum
8	-	Serenade ASO	Serenade @ 7-10 day intervals	
9	-	Rovral WG	Repeat 10-14 days later then @ 7 day intervals to harvest	
10	-	Switch	-	Switch
11	-	Serenade	Trianum P†/Serenade @ 7-14 day intervals	
12	-	Amistar	Trianum P†/Serenade @ 7-14 day intervals	

* Commercially this product should only be used between April & October in protected lettuce.
† Treatments washed off post-application

N.B. The glasshouse area was pre-treated with Contans to minimize the risk of Sclerotinia compromising the trial which was focused on *Rhizoctonia*.

Table 5. Application Rates

Product	Active ingredient	Rate (ha)
Basilex	tolclofos-methyl 50% w/w	20 kg (full), 10 kg (half)
Amistar	azoxystrobin 250g ai/l	1 l
Rovral WG	iprodione 75% w/w	0.033 kg per 100 l water
Signum	boscalid & pyraclostrobin 26.7:6.7% w/w	1.5 kg
Serenade ASO	*Bacillus subtilis*	10 l
Switch	cyprodinil & fludioxonil 37.5:25 % w/w	0.6 kg
Trianum P	*Trichoderma spp.*	3 g/m^2 in 10 l

Crop Diary

1.10.10	Trial area inoculated with *R. solani* (Treatments 2-12)
1.10.10	Pre-planting fungicide applications carried out
1.10.10	Crop planted
5.10.10	2nd spray application (2-3 days post-planting)
18.10.10	3rd spray application. Trianum P applied to Treatments 11 & 12
1.11.10	1st disease assessment carried out.
2.11.10	Spray application
11.11.10	2nd disease assessment carried out
11.11.10	Spray application
3.12.10	Final disease assessment and harvest.

Inoculation with *Rhizoctonia solani*

A virulent strain of *R. solani* previously cultured from infected lettuce was used in this study. The fungus was bulked up using sterile vermiculite (5 l), maize meal (10 g) and 500 ml of sterile distilled water. Approximately half a 90 mm petri dish of an actively growing pure culture was cubed and added to the sterile vermiculite mix. The cultures (1 bag per plot) were maintained at ambient temperatures in the plant pathology laboratory at STC for approximately 2 weeks with regular turning and shaking to ensure even growth of the culture throughout the medium. The active cultures were distributed across the inoculated plots (Treatments 2-12) and lightly raked into the surface soil prior to applying the pre-planting fungicide treatments (Treatments 3-6) and planting the crop. During the planting procedure the uninoculated plots (Treatment 1) were planted first to avoid the movement of *R. solani* on tools, hands or footwear.

Disease assessments

Three full disease assessments were carried out in the crop. The general disease assessment scale shown in the methods for Trial 1 was used. Where other foliar infections were noted the incidence and severity of these organisms was also recorded to provide a full picture of possible control resulting from the fungicide applications. A total of 20 heads per plot were assessed on each date. During the final disease assessment the lettuce were excised at soil level and inverted prior to assessment. Following assessment 20 heads per plot were weighed, trimmed and re-weighed to provide information on yield in this trial.

Residue Data

Lettuces from each treatment were retained (frozen) for residue testing.

Results

Trial 1. Primary Target: *Sclerotinia sclerotiorum*

During the first disease assessment carried out on the 20th October 2009, no plants with *Sclerotinia* bottom-rot were observed, although germinating apothecia (*Sclerotinia* sporing structures) were observed in several (ca. 17%) of the plots. Some evidence of phytotoxicity was observed in the plots treated with Switch (T4) – this took the form of leaf crinkling (see fig. 2) and this was due to an accidental high rate of use (1.5 kg) for the first application.

Figure 2. Phytotoxicity observed in plants treated with incorrect rate of Switch (on left) compared to an untreated lettuce (right).

During the second assessment (3/11/09) some early basal rot infections with *Sclerotinia* were observed (Table 6), though the levels of infection were low and no significant differences were recorded.

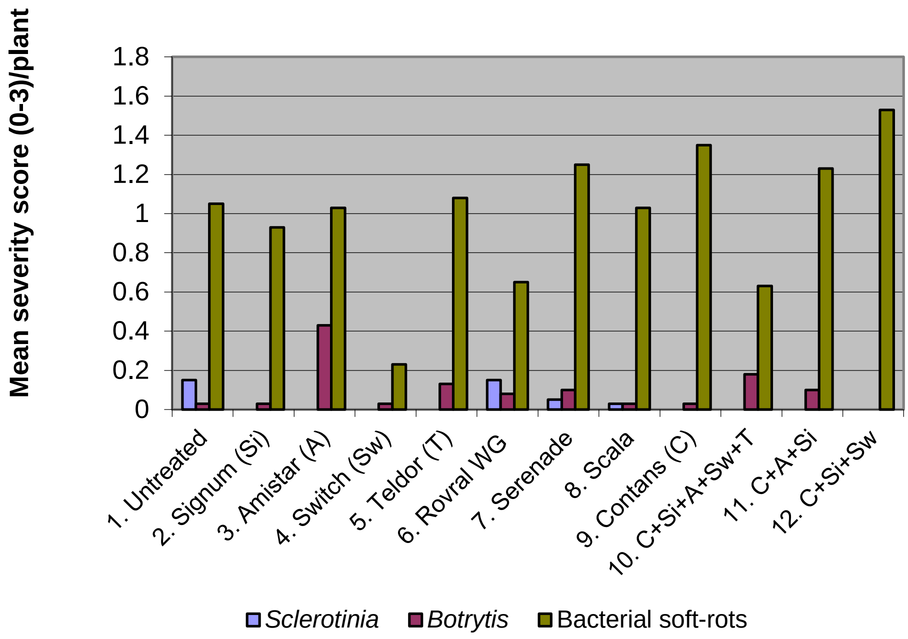
Table 6. Mean severity of *Sclerotinia* on 3/11/09

Treatment No.	**Mean severity of *S. sclerotiorum* (0-3)**
1 Untreated	0.00 a
2 Signum (Si)	0.00 a
3 Amistar (A)	0.00 a
4 Switch (Sw)	0.00 a
5 Teldor (T)	0.00 a
6 Rovral WG	0.12 a
7 Serenade	0.00 a
8 Scala	0.02 a
9 Contans (C)	0.00 a
10 C+Si+A+Sw+T	0.02 a
11 C+A+Si	0.00 a
12 C+Si+Sw	0.00 a
LSD (P=0.05)	*0.09*
Standard Deviation	*0.06*
Co-efficient of Variance	*405.2*

Means followed by the same letter do no significantly differ (P=0.05, Student-Newman-Keuls)

The final disease assessment was carried out on the 1st December 2009. During this assessment the lettuce were cut and inverted prior to assessment, plants were scored for the incidence and severity of *Sclerotinia, Botrytis* and diseases present (Chart 1).

Chart 1. Mean disease incidence and severity/plant during the final assessment (1.12.09)



The data show that the incidence of *Sclerotinia* was very low across the trial and no significant differences in the level of *Sclerotinia* or *Botrytis* were observed between the treatments. There were significantly higher levels of bacterial bottom rots in T9 and T12 compared to T4. This may either be due to unseen damage from the applied treatments or that the other treatments prevented opportunities for bacterial colonisation.

Low light levels during the trial period severely impacted on expected crop development and head weights were lower than anticipated (Table 7). However, as the heads were beginning to deteriorate and bolt slightly by the harvest date and this led to an unusually high level of bacterial soft-rots in the crop.

Table 7. Yield data from Trial 1.

Treatment	Mean untrimmed head weight (g)	Mean trimmed head weight (g)
1. Untreated	171.5 a	143.8 a
2. Signum (Si)	195.8 a	167.3 a
3. Amistar (A)	179.3 a	155.3 a
4. Switch (Sw)	147.0 a	136.8 a
5. Teldor (T)	173.0 a	143.8 a
6. Rovral WG	180.8 a	152.8 a
7. Serenade	173.8 a	146.3 a
8. Scala	172.0 a	142.8 a
9. Contans (C)	175.5 a	142.8 a
10. C+Si+A+Sw+T	164.5 a	143.3 a
11. C+A+Si	184.3 a	155.8 a
12. C+Si+Sw	170.3 a	134.5 a
LSD (P=0.05)	*24.2*	*23.8*
Standard Deviation	*16.7*	*16.5*
Co-efficient of Variance	*9.6*	*11.2*

Means followed by the same letter do no significantly differ (P=0.05, Student-Newman-Keuls)

No significant differences were observed between the mean head weights across the trial, although interestingly, treatments comprising Signum and Amistar had the highest untrimmed and trimmed head weights.

Residue Data

Samples of harvested lettuce from treatments 2, 3, 4, 5, 6, 8, 10, 11 and 12 were retained and sent for pesticide residue analyses to ensure that no maximum residue levels (MRLs) had been exceeded (Table 8).

Table 8. Details of pesticide residue analysis for Trial 1.

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Active ingredients (mg/kg)	Residue/treatment (mg/kg)									
	MRL	**2**	**3**	**4**	**5**	**6**	**8**	**10**	**11**	**12**
azoxystrobin	**3**	<0.01	**0.02**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
boscalid	**10**	**3.5**	**0.01**	<0.01	**0.01**	**0.02**	**0.05**	**0.02**	**0.03**	**0.06**
cyprodinil	**10**	<0.01	**0.01**	**7.5**	**0.07**	**0.09**	**0.01**	**0.08**	<0.01	**5.6**
fenhexamid	**30**	<0.01	<0.01	<0.01	**3.7**	**0.02**	<0.01	**3.3**	**0.02**	**0.02**
iprodione	**10**	<0.01	<0.01	<0.01	<0.01	**1.7**	<0.01	<0.01	<0.01	<0.01
pyrimethanil	**10**	**0.01**	**0.01**	**0.01**	**0.02**	**0.01**	**4.2**	**0.03**	**0.02**	**0.02**
fludioxonil	**10**	<0.01	<0.01	**6.8**	<0.01	**0.07**	<0.01	**0.01**	<0.01	**4.8**
pyraclostrobin	**2**	**0.7**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Figures shown in bold are above the limit of detection

None of the harvested lettuce exceeded the MRL in this study.

Trial 2. Primary Target: *Bremia lactucae*

Downy mildew developed in the inoculated plants and was first observed 15 days post-inoculation on the 14th May 2010 (Fig 3). Sclerotinia also developed as the crop established, presumably from the sclerotial inoculum applied in Trial 1. A full disease assessment was carried out on the 17th May when the incidence and severity of both downy mildew and *Sclerotinia* was assessed (Table 9).

Figure 3. Early development of downy mildew on inoculated guard lettuce

Table 9. Details of an interim disease assessment carried out on the 17th May 2010

Treatment	Mean DM severity/plant (0-3 scale)	Mean *Sclerotinia* severity/plant (0-3 scale)
1 Untreated control	0.05 a	0.00 b
2 Standard programme	0.00 a	0.00 b
3 Revus	0.00 a	0.00 b
4 Signum	0.00 a	0.00 b
5 Serenade	0.00 a	0.02 b
6 Previcur Energy	0.00 a	0.00 b
7 Exp. Prog. 1	0.00 a	0.00 b
8 Exp. Prog. 2	0.02 a	0.15 a
9. Exp. Prog. 3	0.17 a	0.05 b
10 Exp. Prog. 4	0.00 a	0.00 b
11 Exp. Prog. 5	0.00 a	0.00 b
12 Exp. Prog. 6	0.00 a	0.00 b
LSD (P=0.05)	*0.1*	*0.1*
Standard Deviation	*0.1*	*0.1*
Co-efficient of Variance	*499.6*	*321.9*

Means followed by the same letter do no significantly differ (P=0.05, Student-Newman-Keuls)

Downy mildew levels were low in this early assessment. Some treatments remained completely free of infection, whilst others (T1, T8 & T9) showed a slight infection, but no statistically significant differences were observed at this stage in the crop. A significantly higher level of *Sclerotinia* was seen in T8, although again infection was at the early stages.

The final disease assessment and harvest were carried out on the 7th June 2010. Again the crop was assessed for the incidence of the predominant diseases, in this case, of downy mildew and *Sclerotinia* (Table 10).

Table 10. Details of the final disease assessment (7.6.10)

Treatment	**Mean DM severity/plant (0-3 scale)**	**Mean *Sclerotinia* severity/plant (0-3 scale)**
1 Untreated control	0.06 b	2.44 a
2 Standard programme	0.11 b	0.49 d
3 Revus	0.00 b	2.27 ab
4 Signum	0.57 a	0.12 d
5 Serenade	0.25 b	1.52 bc
6 Previcur Energy	0.00 b	1.92 abc
7 Exp. Prog. 1	0.00 b	1.97 abc
8 Exp. Prog. 2	0.21 b	1.26 c
9 Exp. Prog. 3	0.00 b	2.34 a
10 Exp. Prog. 4	0.27 b	0.27 d
11 Exp. Prog. 5	0.31 b	1.52 bc
12 Exp. Prog. 6	0.25 b	0.14 d
LSD (P=0.05)	*0.2*	*0.5*
Standard Deviation	*0.2*	*0.4*
Co-efficient of Variance	*90.7*	*27.4*

Means followed by the same letter do no significantly differ (P=0.05, Student-Newman-Keuls)

Unfortunately, downy mildew disease levels remained rather low and this was attributed to a spell of warmer weather making conditions for infection less favourable. Several of the treatments resulted in complete control of infection (T3 – Revus, T6 – Previcur Energy, T7 – Exp. prog. 1 and T9 – Exp. prog. 3. Both of these experimental programmes featured the use of Revus and Previcur Energy along with Fubol Gold (mancozeb + metalaxyl-M). This result is particularly interesting as whilst Fubol Gold was used at a slightly reduced rate of 1.25 kg/ha rather than the field rate of 1.9 kg/ha, activity does not seem to have been reduced. A significantly higher incidence and severity of downy mildew was seen in T4 – Signum, and this is perhaps a little unexpected as the strobilurin fungicides do tend to have some activity against oomycete fungi.

Figure 4. Sclerotinia in Trial 2 at harvest

A naturally occurring infection with *Sclerotinia* developed in this trial following earlier inoculation of the glasshouse prior to Trial 1. The incidence and severity of infection was moderate to high (Fig 4). Interestingly, some treatment regimes selected for the use in this trial for *Bremia* control did have some efficacy against *Sclerotinia* also. Significantly lower levels of the infection were observed in treatments 2 – Standard Programme, T4 – Signum, T8 -T10 – Exp prog 4 and T12 – Exp prog 6. A common denominator of these treatments is the inclusion of a strobilurin (in Amistar and Signum).

Table 11. Yield data from Trial 2.

Treatment	**Mean untrimmed head weight (g)**	**Mean trimmed head weight (g)**
1 Untreated control	191.7 d	69.5 e
2 Standard programme	333.2 a	262.2 ab
3 Revus	244.0 bcd	138.5 d
4 Signum	296.0 abc	220.0 abc
5 Serenade	247.2 bcd	148.5 cd
6 Previcur Energy	208.0 d	133.0 d
7 Exp. Prog. 1	237.2 cd	134.2 d
8 Exp. Prog. 2	306.7 ab	191.0 cd
9 Exp. Prog. 3	245.0 bcd	150.5 cd
10 Exp. Prog. 4	282.7 abc	207.7 bcd
11 Exp. Prog. 5	279.0 abc	177.2 cd
12 Exp. Prog. 6	341.7 a	277.5 a
LSD (P=0.05)	*42.8*	*50.4*
Standard Deviation	*29.7*	*34.9*
Co-efficient of Variance	*11.1*	*19.9*

Means followed by the same letter do no significantly differ (P=0.05, Student-Newman-Keuls)

There were some highly significant differences in the mean head weights of the untrimmed and trimmed lettuce between treatments compared to the untreated control. These were closely linked with the incidence and control of *Sclerotinia* in the crop rather than any efficacy associated with downy mildew (Table 11). Treatments showing efficacy against *Sclerotinia* resulted in significantly higher mean head weights as would be expected. The standard programme comprising sprays of Amistar, Fubol Gold (reduced rate), Signum and Serenade resulted in a trimmed head weight nearly 4 times higher than untreated controls.

Residue data

Samples of harvested lettuce from treatments 2, 3, 4, 6, 7, 8, 9, 10, 11 and 12 were retained and sent for pesticide residue analyses to determine whether maximum residue levels (MRLs) had been exceeded (Table 12).

Table 12. Details of pesticide residue analysis for Trial 1.

Active ingredients (mg/kg)	**Residue/treatment (mg/kg)**										
	MRL	**2**	**3**	**4**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
mancozeb*	**5**	**0.45**	-	-	-	<0.05	-	<0.05	<0.05	<0.05	<0.05
boscalid	**10**	**1.2**	-	**0.51**	-	-	<0.05	-	-	**0.32**	<0.05
pyraclostrobin	**2**	**0.26**	-	**0.07**	-	-	<0.01	-	-	**0.05**	<0.01
mandipropamid	**25**	-	**0.16**	-	-	**0.12**	-	<0.01	-	-	-
propamocarb-HCl	**50**	-	-	**0.008**	**4.9**	-	-	**0.87**	**2.0**	-	**0.05**
phosphonic acid	-	-	-	-	**25**	-	-	**2.6**	**7.0**	-	**1.9**
fosetyl-aluminium	**75**	-	-	-	<0.5	-	-	<0.5	<0.5	-	<0.5
metalaxyl-M	**2**	<0.02	-	-	-	<0.02	-	<0.02	<0.02	<0.02	<0.02
azoxystrobin	**3**	<0.05	-	-	-	-	-	-	<0.05	<0.01	-
cyprodinil	**10**	-	-	-	-	-	-	-	**0.02**	**0.26**	**0.03**
fludioxonil	**10**	-	-	-	-	-	-	-	**0.01**	**0.17**	**0.02**

Figures shown in bold are above the limit of detection.
* As total dithiocarbamates
- Not tested

None of the harvested lettuce exceeded the MRL in this study.

Trial 3. Primary Target: *Rhizoctonia solani*

Early bottom-rot symptoms were observed in the crop approximately 12-15 days post-planting. The early symptoms were visible as discoloration of the basal leaves close to the main stem. The first disease assessment was carried out on the 1st November 2010 with a second assessment on the 11th November. Plants were scored for the incidence and severity of *Rhizoctonia* bottom-rot (Table 13).

Table 13. Details of the first and second disease assessments (1 & 11th November 2010)

Treatment No.	Mean *Rhizoctonia* severity/plant (0-3 scale)	
	1.11.10	**11.11.10**
1 uninoculated control	0 b	0.11 b
2 inoculated control	0.45 a	1.07 a
3 Basilex pre-planting	0.05 b	0.15 b
4 Amistar pre-planting	0.01 b	0.10 b
5 Basilex (half) / Amistar	0.01 b	0.07 b
6 Amistar / Rovral	0.02 b	0.22 b
7 Signum / Signum	0.02 b	0.05 b
8 Serenade / Serenade	0.26 ab	0.47 b
9 Rovral / Rovral	0.20 ab	0.39 b
10 Switch / Switch	0.10 b	0.26 b
11 Serenade / Trianum	0.24 ab	0.36 b
12 Amistar/Trianum/Serenade	0.02 b	0.07 b
LSD (P=0.05)	*0.2*	*0.4*
Standard Deviation	*0.1*	*0.3*
Co-efficient of Variance	*123.1*	*109.1*

Means followed by the same letter do no significantly differ (P=0.05, Student-Newman-Keuls)

The results of the early assessments demonstrate that the pathogen was developing successfully and infecting plants as intended. Significantly higher levels of infection were seen in the inoculated control (T2) compared to the uninoculated control. The plots had received pre-planting fungicide applications of Amistar or Basilex (T3-T6) maintained slightly better control of infection than other treatment regimes. Additionally, Signum applied post-planting was as effective as the pre-planting applications of Basilex and Amistar.

During the final assessment carried out on the 3rd December 2010 plots were assessed for the incidence and severity of *Rhizoctonia*, *Botrytis* and *Sclerotinia* (Table 14).

Table 14. Disease incidence during the final disease assessment (3/12/10)

Treatment No.	Mean *Rhizoctonia* severity/plant (0-3 scale)	Mean *Botrytis* severity/plant (0-3 scale)	Mean *Sclerotinia* severity/plant (0-3 scale)
1 uninoculated control	0.51 b	0.01 b	0.13 a
2 inoculated control	1.42 a	0.01 b	0.09 a
3 Basilex pre-planting	0.79 ab	0.0 b	0.21 a
4 Amistar pre-planting	0.90 ab	0.0 b	0.0 a
5 Basilex (half) / Amistar	0.45 b	0.04 b	0.0 a
6 Amistar / Rovral	0.74 ab	0.0 b	0.0 a
7 Signum / Signum	0.49 b	0.11 a	0.0 a
8 Serenade / Serenade	1.06 ab	0.01 b	0.16 a
9 Rovral / Rovral	1.14 ab	0.02 b	0.04 a
10 Switch / Switch	0.95 ab	0.0 b	0.05 a
11 Serenade / Trianum	1.12 ab	0.0 b	0.03 a
12 Amistar/Trianum/Serenade	0.65 ab	0.01 b	0.01 a
LSD (P=0.05)	*0.5*	*0.1*	*0.2*
Standard Deviation	*0.4*	*0.0*	*0.1*
Co-efficient of Variance	*44.5*	*233.3*	*202.1*

Means followed by the same letter do no significantly differ (P=0.05, Student-Newman-Keuls)

The results of the final assessment indicate that all the applied treatments were effective and reduced the level of infection by *Rhizoctonia* compared to the inoculated control. The level of control achieved with T5 and T7 was significantly better than the inoculated control. In T5 Basilex was applied at half label rate pre-planting with a single application of Amistar 7-14 days post-planting, this treatment regime appears to be more effective than T3 where a single full-dose rate of Basilex was applied pre-planting. Treatment 7 received 2 applications of Signum (at 2-3 days post-planting and again at 14-28 days post-planting). Both of these treatment regimes reduced the amount of infection to below that recorded in the uninoculated control. Many of the other treatment regimes also resulted in a moderate - good level of *Rhizoctonia* control (see figs. 5-7).

Botrytis and *Sclerotinia* infections were low overall in this study with no significant differences between the level of infection between the treatments, the latter possibly associated with the pre-planting application of Contans in the glasshouse.

The collected yield data (Table 13) indicates that the mean head weight of lettuce from all the treated plots and the uninoculated plots (T1) were heavier than the inoculated control lettuce where the highest levels of *Rhizoctonia* bottom-rot was recorded. Lettuce from T6 (Amistar followed by Rovral) gave significantly heavier untrimmed head weights than the inoculated control treatments (T2). Several treatments (T3-7, 10 and 12) gave a significantly higher trimmed head weight compared to the inoculated control.

Table 15. Yield data from trial 3

Treatment No.	Mean untrimmed head weight (g)	Mean trimmed head weight (g)
1 uninoculated control	105.1 ab	81.5 a
2 inoculated control	81.1 b	51.0 b
3 Basilex pre-planting	101.9 ab	78.2 a
4 Amistar pre-planting	108.7 ab	85.0 a
5 Basilex (half) / Amistar	111.4 ab	93.5 a
6 Amistar / Rovral	116.6 a	93.4 a
7 Signum / Signum	112.9 ab	89.1 a
8 Serenade / Serenade	92.4 ab	66.2 ab
9 Rovral / Rovral	96.2 ab	70.0 ab
10 Switch / Switch	107.5 ab	80.9 a
11 Serenade / Trianum	103.7 ab	70.4 ab
12 Amistar/Trianum/Serenade	104.4 ab	83.4 a
LSD (P=0.05)	*18.9*	*17.3*
Standard Deviation	*13.1*	*11.9*
Co-efficient of Variance	*12.6*	*15.2*

Means followed by the same letter do no significantly differ (P=0.05, Student-Newman-Keuls)

Residue Data

Table 16. Details of pesticide residue analysis for Trial 3.

Active ingredients (mg/kg)	**Residue/treatment (mg/kg)**							
	MRL	**3**	**4**	**5**	**6**	**7**	**9**	**10**	**12**
boscalid	**10**	**-**	-	-	-	**3**	-	-	-
pyraclostrobin	**2**	**-**	-	**-**	-	**1.2**	-	-	-
azoxystrobin	**3**	-	<0.05	**0.1**	<0.05	-	-	-	<0.05
tolclofos methyl	**2**	**0.27**	-	**0.14**	-	**-**	-	-	-
iprodione	**10**	-	-	**-**	**3.9**	**-**	**5.7**	**-**	**-**
cyprodinil	**10**	-	-	-	**-**	-	-	**0.91**	**-**
fludioxonil	**10**	-	-	-	-	-	-	**1.3**	-

Figures shown in bold are above the limit of detection.

None of the harvested lettuce exceeded the MRL in this study.

Figure 5. Rhizoctonia bottom-rot T2

Figure 6. Low level of bottom-rot T7

Figure 7. Low level of bottom-rot T12

Discussion

The set of 3 trials carried out during this study allowed STC to devise a range of spray programmes, using some of the newly approved fungicides and also some active ingredients which may soon be approved. The programmes were designed to investigate the efficacy of the products to control *Sclerotinia*, *Bremia* and *Rhizoctonia solani* in 3 separate artificially inoculated trials under protection.

Although infection with *Sclerotinia* was relatively low in trial 1, a slight infection was observed in T1 (untreated), T6 (Rovral) and T7 (Serenade). The other products used singly or as part of programmes, particularly when used following a pre-planting treatments, appear to have been effective, although further trials data following a higher level of infection would provide a greater level of confidence in this result. Low levels of *Botrytis* were also observed in this study, though no control of the naturally occurring infection was observed. Treatment 4 (Switch) appeared to give the best control of all basal rot infections and resulted in the best plots of lettuce despite the slight crop damage resulting from an application of the inappropriate rate of product early in the trial. The lettuce remained generally free of all basal rot infections.

The second trial, carried out in the same glasshouse was carried out primarily to investigate the control of downy mildew which was artificially inoculated into the guard plants in the trial area. Low levels of downy mildew infection were observed in some of the treatments, and some products, Previcur Energy, Revus and Fubol Gold, appeared to provide some control. Fubol Gold was used at the slightly lower application rate of 1.25kg/ha in this study with no obvious reduction in efficacy. A significantly higher rate and severity of downy mildew infection was seen in the plots treated with Signum (T5) compared to the other treatments though this cannot be fully explained. The pyraclostrobin element of this product would be expected to give some degree of control, although previously reported resistance issues regarding downy mildews and strobilurin products cannot be discounted. A moderate, naturally occurring infection with *Sclerotinia* also developed in this study, and provided an opportunity to gather additional data. All of the applied treatments reduced infection compared to the untreated control, however significantly lower levels of infection were observed in T2, T4, T8, T10 and T12. Although none of these treatments, or programmes were specifically designed to control *Sclerotinia*, many of the products used (Amistar, Fubol Gold, Signum, Serenade and Switch) were observed to have measurable efficacy against this pathogen. This is very useful information to help design spray programmes to minimize chemical applications.

In the final trial carried out during the autumn of 2010 the efficacy of a number of products and programmes to control *Rhizoctonia* bottom-rot were investigated. Pre-treatment of the trial area with an application of Contans appeared to provide good control over the development of *Sclerotinia* following the 2 previous trials and reduced the impact of this pathogen on the trial crop. The pre-planting inoculation with *Rhizoctonia solani* provided a moderate to good level of infection in the study, since the highest levels of infection were observed in the inoculated control plots (T2). Treatments 3-6 focused mainly on the use of pre-planting treatments with Basilex or Amistar. Although all of these treatments reduced the level of infection compared to the inoculated control (T2), T5 (half-rate Basilex followed by an application of Amistar 14 days later) resulted in a significant reduction of infection in those plots and this could provide a workable alternative to the use of OPs. Two applications of Signum (T7) also gave excellent control of *Rhizoctonia* bottom-rots in this study. Effectively this approach may provide an opportunity to eliminate the use of the OP Basilex altogether. However, repeated use of a single mode of action product may lead to an increased resistance risk so it may not be entirely appropriate. The data however does show that potentially an alternative strategy can be devised for *Rhizoctonia* control and this requires further investigation. The data also suggests that the use of biological control agents alone e.g. T8 (Serenade) and T11 (Serenade/Trianum) are less effective against *Rhizoctonia*. However, where they are used as later treatments following an earlier application of a conventional fungicide e.g. T12 (Amistar followed by Serenade and Trianum P) the biological control products were able to maintain control of infection, whilst at the same time reducing the risk of residue problems on crops. This may be of considerable benefit particularly in the longer winter lettuce crops where residue issues arise more frequently.

Conclusions

The trials carried out as part of this project have provided some valuable information on the use of products recently approved for use on protected lettuce, as well as some that may gain approval in the near future. Useful data was collected on 2 out of the 3 pathogens under investigation, although disappointingly little data was collected on *Bremia* control, and this must be considered to be due to weather conditions experienced during the relatively short duration of the project.

The work has identified a number of products and programmes which appear to offer a reasonable degree of control of some of the key pathogens on lettuce. For example Previcur Energy, Fubol Gold (at a reduced application rate) and Revus appeared to provide some control of downy mildew in the second trial, whilst additional data gathered on basal infections suggested that the application of Switch was also beneficial. Contans applied pre-planting appeared to provide good control of *Sclerotinia* in the final trial though this cannot be confirmed as the prevailing weather may have attenuated its development. The severity of *Rhizoctonia* was significantly reduced in plots treated with Basilex applied at half the normal rate when followed by Amistar 14 days later. Signum also proved very effective in this trial and this could be very important when combined with *Sclerotinia* control. Potentially the strobilurin fungicides may eliminate the need for use of the OP fungicide Basilex. However it would be necessary to identify an alternative partner fungicide for resistance management purposes.

It is hoped that results from this study will enable growers of protected lettuce to develop effective routine spray programmes for fungal pathogen control. There is now a range of products with proven efficacy against these diseases which should enable growers to rotate products and potentially use some of them below-label rates to deliver cost savings. Data collected also indicate that the biological control products used as late applications in a programme maintained disease control, thus reducing the need for chemical fungicide applications close to harvest which could result in MRL exceedance issues.

It is hoped that the protected lettuce industry find the results of these trials useful, and that they can benefit not just from this short-term set of trials, but also see the value of this type of study where 'disease nurseries' provide an opportunity to trial new and existing products on a rolling programme repeated bi-annually or annually as required.

Knowledge and Technology Transfer

26th January 2010 – Cathryn Lambourne attended Protected Lettuce technology group meeting and discussed results to-date

HDC article – February 2011.

Appendix 1
Plan – Trial 1

HDC – Best Practice for Disease Control in Lettuce

E480 2009 Fairfield 6

P6 T11	P12 T10	P18 T6	P24 T5	P30 T9	P36 T3	P42 T7	P48 T8
P5 T2	P11 T5	P17 T3	P23 T7	P29 T4	P35 T1	P41 T11	P47 T10
P4 T8	P10 T7	P16 T9	P22 T11	P28 T12	P34 T6	P40 T5	P46 T4
P3 T6	P9 T3	P15 T1	P21 T12	P27 T2	P33 T10	P39 T12	P45 T9
P2 T4	P8 T9	P14 T10	P20 T8	P26 T11	P32 T7	P38 T2	P44 T1
P1 T1	P7 T12	P13 T2	P19 T4	P25 T5	P31 T8	P37 T3	P43 T6

DOOR

P A T H

Treatments

1.Untreated
2. Signum (Si)
3. Amistar (A)
4. Switch (Sw)
5. Teldor (T)
6. Rovral WG
7. Serenade
8. Scala
9. Contans (C)
10. C+Si+A+Sw+T
11. C+A+Si
12. C+Si+Sw

Plot Dimensions

1.1m x 1.7m

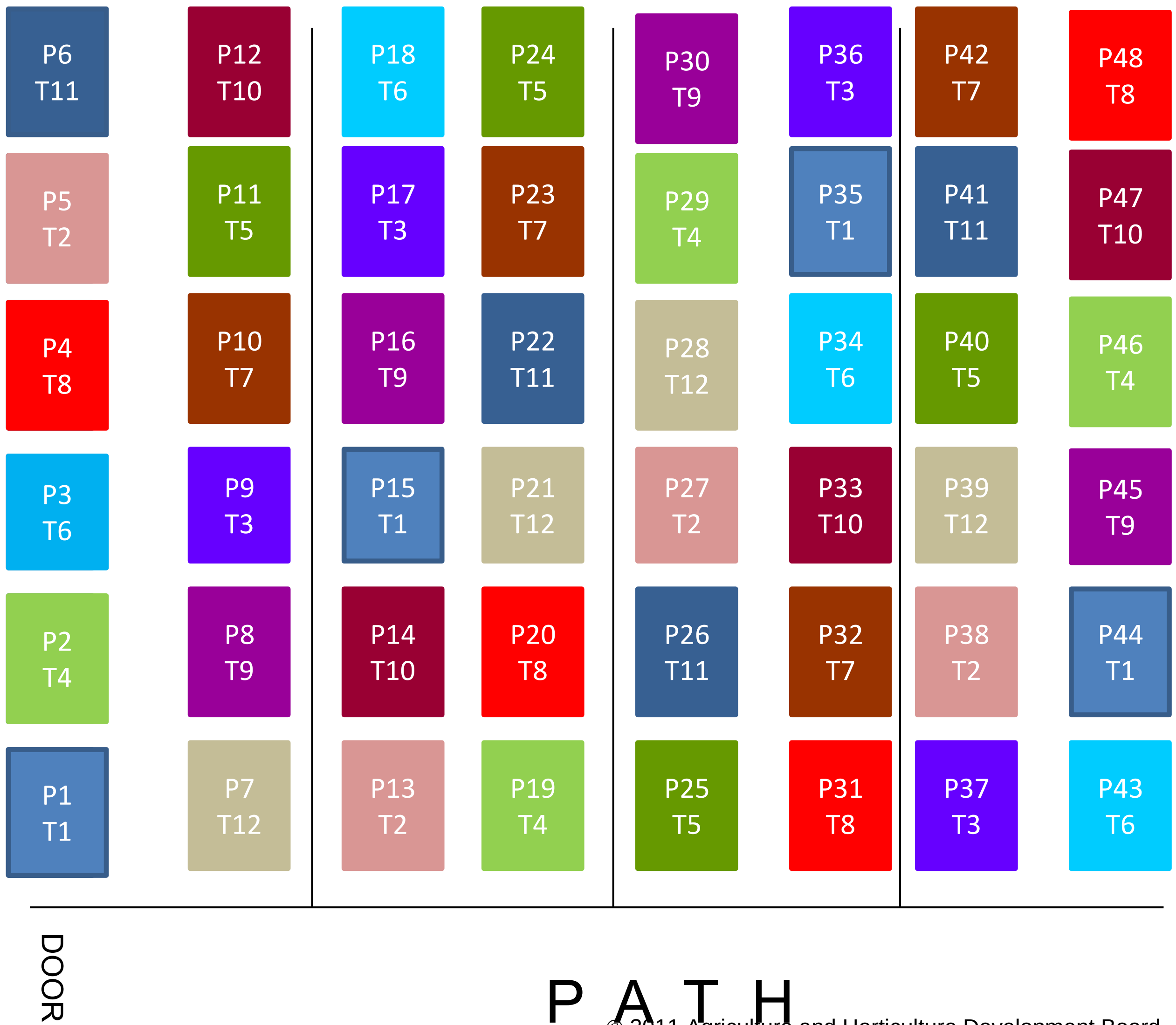
Var: Wiske - Butterhead

N

Plan – Trial 2

HDC – Best Practice for Disease Control in Lettuce

Trial 2 – Bremia control E480 2010 Fairfield 6



Treatments

1. Untreated
2. Am/FG/Sig/Ser
3. Revus
4. Signum
5. Serenade
6. Previcur Energy
7. FG/Rev (TM)
8. Valbon/Sig/Phos/Phos
9. Rev/FG/PE/Serenade
10. Am/FG (TM)/PE/Sw (TM)/Ser
11. FG+Rev/Ser/Sig+Sw/Ser
12. PE+Sig/FG+Sw/Ser/Ser

Plot Dimensions

1.3m x 1.3m (6 x 7 plants)

Var: Cobham Green - Butterhead

← N

Plan – Trial 3

HDC – Best Practice for Disease Control in Lettuce

Trial 3 – Rhizoctonia control   E480   2010   Fairfield 6

P6 T10	P12 T3	P18 T4	P24 T12	P30 T2	P36 T1	P42 T4	P48 T5
P5 T7	P11 T6	P17 T8	P23 T3	P29 T5	P35 T9	P41 T6	P47 T12
P4 T1	P10 T8	P16 T6	P22 T5	P28 T10	P34 T6	P40 T11	P46 T9
P3 T5	P9 T12	P15 T2	P21 T11	P27 T8	P33 T7	P39 T10	P45 T1
P2 T9	P8 T2	P14 T7	P20 T9	P26 T11	P32 T3	P38 T3	P44 T2
P1 T4	P7 T11	P13 T10	P19 T1	P25 T12	P31 T4	P37 T7	P43 T8

DOOR

P A T H

N

Treatments

1.Uninoculated, untreated

2. Inoculated, untreated

3. Basilex pre-planting

4.Amistar pre-planting

5.Basilex ½ N pre-planting

6. Amistar pre-planting/Rovral

7.Signum

8.Serenade

9.Rovral

10.Switch

11.Serenade/Trianum P (repeated)

12.Amistar/ (Serenade/Trianum P) repeated.

Plot Dimensions

1.3m x 1.3m (6 x 7 plants)

Var: Trinette