Project title:	Lettuce: Further development of 'Best Practice' for disease control in protected and outdoor crops
Project number:	FV/PE 410
Project leader:	Dr G M McPherson
Report:	Annual Report 2013
Previous report:	None
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Date project commenced:	01 August 2012
Date project completed (or expected completion date):	31 July 2015

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

AUTHENTICATION

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

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GROWER SUMMARY

Headline

Using tank mixes of fungicides with different modes of action (often at half rates) can reduce the risk of residues at harvest and provide disease protection later in the season in protected and outdoor lettuce.

Background

Downy mildew (caused by the pathogen *Bremia lactucae)* is responsible for most losses in both outdoor and protected lettuce. Soil-borne diseases, such as *Sclerotina* and *Rhizoctonia* are also important and contribute to significant losses in some field and glasshouse crops, though interestingly the latter pathogen only appears to be problematic under protection. White mould (caused by *Sclerotinia* sp.*)* causes a severe head decay, especially near maturity and bottom rot (caused by *Rhizoctonia solani*) can also be very damaging though, as indicated, particularly in protected lettuce crops. Grey mould (caused by the pathogen *Botrytis cinerea*) is very often present on the oldest leaves and is usually removed during the normal harvest trimming, but in wet seasons heavy infections can reduce head weight as more leaves need to be removed.

The primary purpose of the project is to identify a range of novel fungicides and bio-control products with activity against the primary pathogens mentioned above but also taking due regard of any 'incidental' control of more minor sporadic pathogens. The main aim is to evaluate a series of spray programmes which provide broad activity on the crop which also provide a reduced risk of residues at harvest and which ensure minimal risk of resistance development.

Summary

The first outdoor (ADAS) and protected (STC) trials were completed in autumn 2012.
In the outdoor lettuce trial (Figure 1) there were 16 treatment programmes at four application timings and downy mildew was the prevalent disease with *Botrytis* affecting plants secondarily. Other pathogens, if present, were at low to trace levels only. The trial site was on a commercial farm so it was not realistic to artificially introduce the pathogens. There were significant differences between treatments for the control of downy mildew. Four of the treatment programmes looked particularly promising. Encouragingly, the most effective programmes for downy mildew control were based on products already approved for use on lettuce e.g. Amistar (azoxystrobin), Karamate (mancozeb), Signum (boscalid +

pyraclostrobin), Fubol Gold (mancozeb + metalaxyl M), Revus (mandipropamid), Switch (cyprodinil + fludioxonil), Previcur Energy (fosetyl-aluminium + propamocarb hydrochloride) and two other, experimental coded products – F145 and F150. There were no significant differences between treatment programmes for the control of *Botrytis*. There were no significant differences between treatment programmes for trimmed head weight after harvest. All pesticide residues remained below the limit of detection.

Figure 1. Autumn outdoor trial, Norfolk.

The protected trial was done in a glasshouse which had been used in the past for lettuce disease trials and which was known to have high levels of fungal pathogens, especially *Sclerotinia* and *Rhizoctonia,* already present in the soil. In this trial there were 12 treatment programmes at four application timings. The treatments included an untreated, an industry standard, four commercial programmes, four experimental programmes, a straight conventional experimental (coded) active and a straight biological experimental (coded) product.

Downy mildew and *Botrytis* infected the crop early and *Sclerotinia* developed at moderate to severe levels, therefore no artificial inoculation, as planned, was required. However, and

somewhat surprisingly, the levels of *Rhizoctonia* recorded were low, given the previous cropping known problems with *Rhizoctonia* bottom rot and absence of soil sterilisation.

There were significant differences between treatments when assessed for downy mildew, *Sclerotinia* and the number of dead plants at each assessment date. There were no significant differences between treatments when assessed for *Botrytis* or *Rhizoctonia*. *Sclerotinia* was responsible for most of the plant deaths.

In terms of developing effective fungicide programmes to control such a broad range of target pathogens this initial trial has already demonstrated the challenges faced. For example, the treatments that performed best for control of Downy mildew did not perform well against *Sclerotinia* or *Botrytis*. The treatments that performed best for control of *Sclerotinia* were relatively poor for Downy mildew or *Botrytis* control and the treatments that were most effective against *Botrytis* were less effective against downy mildew or *Sclerotinia*. Therefore, in order to deliver a broad and effective treatment programme, it is appropriate to develop either tank mixes with different active ingredients (included at reduced rates to keep overall cost down) to maintain broad spectrum protection throughout or to tailor the fungicide programme based on climatic factors and relative to disease risk.

In this first study, the standard commercial programme (Amistar/Fubol Gold/Teldor/Revus) provided best control of downy mildew, but it performed poorly against *Botrytis* and below average against *Sclerotinia*. One of the commercial programmes (Fubol Gold/Signum/Switch/Serenade) provided the best overall control of the three pathogens present in this study, and three of the experimental programmes performed reasonably well against all diseases. Disease levels, predominantly *Sclerotinia*, in the glasshouse were so high by the end of the trial that most of the plants in each plot died or were severely diseased, leaving insufficient heads for samples to be taken for residue analyses.

Lab-based screening tests for active ingredients, including new SDHI's, with activity against downy mildew, *Botrytis*, *Rhizoctonia*, *Sclerotinia sclerotiorum* and *S. minor* identified a number of active ingredients capable of inhibiting pathogen growth. Many of the SDHIs provided good to excellent inhibition of *Rhizoctonia* and *Sclerotinia*, but a little surprisingly, were less effective against *Botrytis*. Some products inhibited *Botrytis* growth as well as *Rhizoctonia* (Rovral, iprodione) (Figure 2 (a) & (b)), and *Sclerotinia* (Octave, prochloraz) (Figure 2 (c) & (d)). HDC F158 inhibited all three pathogens, but was most effective against *S. minor*. Fungicides containing metalaxyl and dimethomorph provided good inhibition of *Phytophthora*, an oomycete organism used to represent *Bremia* which cannot be cultured *in*

vitro. Infinito (fluopicolide + propamocarb hydrochloride) also inhibited oomycete growth well. Alternatives to metalaxyl are needed as resistance to this active in downy mildews is well documented.

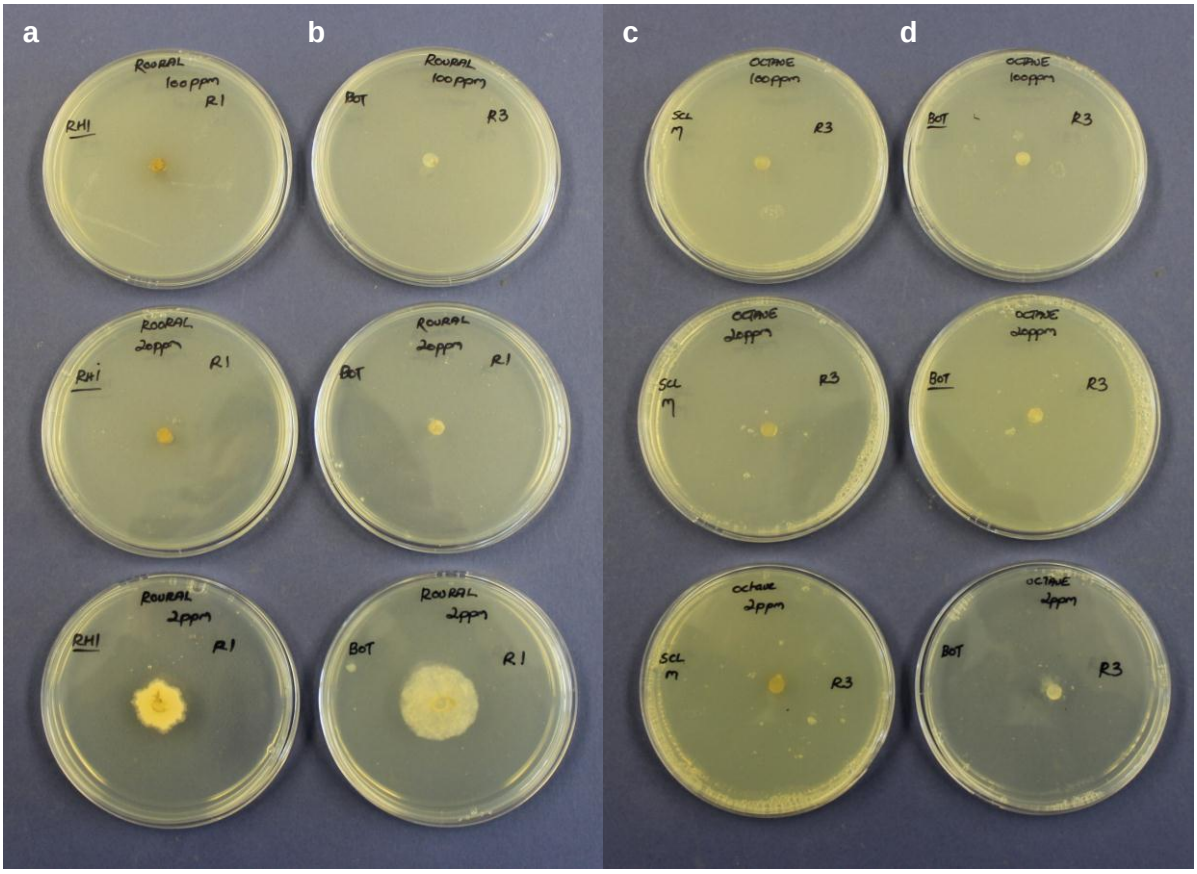

Figure 2. (a) Inhibition of growth of *Rhizoctonia* mycelium on agar plates by Rovral (iprodione). (b) Inhibition of growth of *Botrytis* mycelium on agar plates by Rovral (iprodione). (c) Inhibition of growth of *Sclerotinia* mycelium on agar plates by Octave (prochloraz). (d) Inhibition of growth of *Botrytis* mycelium on agar plates by Octave (prochloraz). The highest concentration of product (100ppm) is at the top of the photograph, followed by 20ppm in the centre and the lowest concentration (2ppm) is at the bottom.

A commercial crop of iceberg lettuce of cultivar Robinson was used for the spring outdoor trial. Pathogen infection was by natural occurrence, and the likelihood of infection was increased by using a field with a history of *Sclerotinia* and crop covers during the early part of the season because of the cold spring. There were 16 treatments combining tank mixes and single product applications. Four post-planting treatment applications were made. There was a high incidence, and moderate severity of *Botrytis* in the trial, and low levels of *Sclerotinia*. No downy mildew or ringspot was recorded in this trial. There was significantly more *Botrytis* in treatments that received Signum at the first application. *Sclerotinia* disease levels were low and no treatment differences were significant. Treatment 10, which contained products for downy mildew control at each application and HDC F151 in a tank mix at the second application, had a significantly lower incidence of *Botrytis* and a lower

Botrytis severity than all the other treatments. At harvest, levels of *Botrytis* were very close to causing losses from extra trimming of the heads. This experiment suggests that good control of *Botrytis* may be difficult to achieve, though there may be scope to maintain protection by using suitable fungicides at the fourth application. No pesticide residues were detected in any of the samples and all remained below the limit of detection.

In the spring protected trial there were 12 treatment programmes including an untreated control (Figure 3). Four post-planting application timings were planned, but only three could be made as the crop matured quickly. The treatments included an untreated, an industry standard, two commercial programmes, four experimental commercial programmes and four experimental (non commercial) programmes. Many of the programmes included Amistar to control *Rhizoctonia* so that they could be compared to the use of Basilex pre-planting which was used in the industry standard treatment. The programmes in this trial were designed to see how late fungicide applications could be made before harvest without incurring residue exceedances. Currently the majority of the fungicide applications are made in the first three to four weeks after planting, exposing the crop to disease infections later on which could make heads unmarketable. Growers are cautious of applying fungicides close to harvest because they do not wish to exceed maximum residue limits (MRLs). These programmes were designed to space out the number of applications to give better control of fungal pathogens from planting to harvest and, by using half rates and tank mixes thus trying to minimise residues at harvest. The crop matured faster than expected so the final treatment applications could not be applied. The crop had to be harvested before the minimum recommended harvest intervals had been reached for many of the products. This enabled data to be gathered on whether reducing application rates also reduced residues at harvest.

Figure 3. Spring protected trial at STC showing plots in the foreground that suffered from severe *Sclerotinia* and *Rhizoctonia* infections.

The variety used was a butterhead lettuce of cultivar Tahamata. To increase the chances of infection by the target pathogens, the trial was done in a glasshouse which had been used in the past for lettuce disease trials and it was known to have high levels of fungal pathogens, especially *Sclerotinia*, already present in the soil. *Rhizoctonia* was artificially introduced by inoculating the soil pre-planting. *Bremia lactucae* was artificially inoculated by applying a spore suspension to six plants per plot on two occasions during the trial. However, neither inoculation with *Bremia lactucae* worked. *Botrytis cinerea* occurred naturally, without artificial infection.

Some treatment programmes included pre-planting applications. The first foliar applications were carried out 2-3 days post-planting, with other applications made at 14 day intervals.

No *Bremia lactucae* was observed in the trial. There were quite high levels of *Botrytis* and moderate levels of *Rhizoctonia* and *Sclerotinia*. The presence of *Botrytis* was not consistent from one assessment to the next, and although there were significant differences between treatments in the first and last assessments, these differences were not repeated in both assessments. Botrytis incidence in the untreated control was low, but may have been masked by the high levels of *Rhizoctonia* and *Sclerotinia* present. There were

significant differences between the levels of *Rhizoctonia* and *Sclerotinia* at all assessments and these differences remained fairly consistent from one assessment to the next. There were low levels of bacterial rot to the lower leaves recorded at harvest.

Some low levels of pesticide residues were recorded at the end of the trial, but these were below the MRLs with the exception of HDC F152, which has an MRL in lettuce of 0.01 mg/kg anyway (the lowest limit of detection). Considering the crop was cut before the minimum harvest interval, the policy of using half rates in tank mixes has meant that products could potentially (subject to appropriate authorisation) be applied closer to harvest when used at lower rates, without appearing to compromise efficacy.

Treatment 3 (Commercial) – (Contans/Amistar/Fubol Gold/Paraat), treatment 6 (experimental commercial tank mixes) - (Amistar + Fubol Gold/Signum + Switch/Paraat + Rovral), and treatment 7 (experimental commercial tank mixes) - (Amistar + Fubol Gold/ Signum + Paraat) resulted in significantly fewer dead plants at the end of the trial than the industry standard. There were differences in the disease severity between these treatments and the standard, but these were not significant. The mean head weight for these treatments was slightly below that recorded for the standard programme, but not significantly so. The number of marketable heads was significantly greater in these treatments than in the standard (Figure 4).

All three programmes had three products in common: Amistar, Fubol Gold and Paraat. Interestingly in plate tests azoxystrobin, the active ingredient of Amistar, did not provide good inhibition of *Rhizoctonia* and *Sclerotinia*, but it is known that some products provide additional activity *in vivo* e.g. the 'turning on' of host defence systems or leaf greening and these effects are not measurable during *in vitro* studies. Contans, which provided good inhibition of *Sclerotinia* in *in vitro* tests, may have helped control *Sclerotinia* in Treatment 3 and Signum, which provided good inhibition of *Rhizoctonia* and *Sclerotinia* in *in vitro* tests, may have helped to control these diseases in treatments 6 and 7, but it was not applied until later in the treatment programmes, as was Rovral in treatment 6, which does not explain why very low levels of these pathogens were recorded in earlier assessments. Treatment 7 only received two treatment applications in total, and yet was one of the best performing treatments. It seems possible that there may be an interaction between Amistar and Fubol Gold, when made as an early application, which is controlling these pathogens more effectively. These results suggest that by using these products in the effective tank mixes at the correct timings, it may not be necessary to use Basilex as a pre-planting treatment. As no *Bremia* infected the trial it is not possible to evaluate the performance of Fubol Gold,

although in the field trial it performed well at controlling the pathogen in treatment programmes that also included Amistar. Such mixtures or alternating programmes will continue to be important to reduce the risk of resistance in the *Bremia* population. Paraat was also used in the field trial programmes and provided quite good control of *Bremia*, although not as good as Fubol Gold.

Figure 4. Spring 2013 protected trial: standard treatment (left) compared to treatment 7 (right). Photos taken at harvest and heads turned over to show condition of lower leaves.

None of the experimental programmes performed as well as the standard or any of the commercial programmes. Whilst this is disappointing, it does suggest that it may be possible to control these important pathogens using existing approved products available to growers without necessarily waiting for new products to be registered and approved.

Knowledge acquired from the first year trials will be used to devise more specific programmes to target these pathogens and refine the treatment applications in the final year of the project.

Financial Benefits

Some useful initial benefits of the project work are the indication that a reduced number of treatment applications could be made per crop by improving timings of application. The use of effective tank mixes of products at reduced rates means that disease control can be maintained and products could potentially be applied closer to harvest. This could result in cost reductions for products and application time. As fungicides could also be applied closer to harvest, crop losses could also be reduced therefore increasing the economic yield. Further work would be required to ensure such uses stay within the regulatory framework.

Action Points

- Design specific spray programmes, using already approved products, based on:
    - the likely risk of pathogens at that time of year
    - the type/cultivar of lettuce grown
    - the cropping history of the site
- There is potential to use reduced application rates of products either in tank mixes or as alternating spray programmes to target 2 or more pathogens simultaneously. Prior to doing this it will be important to check the regulatory situation especially in relation to applications closer to harvest as several products have specific restrictions relating to latest time of application.
- Apply products at timings likely to have the most effect on prevalent pathogens.

SCIENCE SECTION

Introduction

Lettuce (*Lactuca sativa* L.) is the most widely grown outdoor salad crop in the UK with a production area of 5,349 ha for all types of lettuce. In 2010, 157,700 tonnes of lettuce were produced at an estimated value of £84.7 million (Defra Horticultural Statistics). There are five main types of lettuce; crisphead (mostly iceberg), romaine (cos), butterhead (round), leaf and babyleaf. Leafy types take many forms (oak leaf, lollo rosso etc) and include both green and red colours. Crisphead lettuce forms the major type of lettuce grown in the UK, of which iceberg is the most widely grown type (Figure 2).

Figure 5. Commercial Iceberg lettuce crop with downy mildew symptoms on outer leaves.

Downy mildew (*Bremia lactucae)* is a major and potentially devastating disease in protected and outdoor lettuce, particularly so in iceberg varieties, especially when favourable wet, cool, humid conditions prevail. If not prevented or controlled from spreading whole crops can become unmarketable if the disease reaches the head of the lettuce. Where whole fields are lost or ploughed in due to severe outbreaks of the disease losses can reach into

hundreds of thousands of pounds. A hectare of lettuce can be worth £67,000 (Defra Horticultural Statistics 2010).

Other diseases are also important and contribute to significant losses in some crops. Grey mould (*Botrytis cinerea*) is very often present on the oldest leaves and is usually removed during the normal harvest trimming. Occasionally it causes plant losses in young plants or severe basal rots when there are problems at plant establishment. *Sclerotinia sclerotiorum* and *S. minor* cause severe head rots near maturity. Bottom rot caused by *Rhizoctonia solani* is more prevalent on protected crops than in field lettuce. Ringspot (*Microdochium panattonianum*) is easily overlooked and can affect patches in field and protected crops given prolonged wet conditions. In glasshouse crops it is occasionally found in wetter parts of the crop e.g. under leaky gutters.

Even low levels of disease can reduce yield as infected leaves will require extra trimming, so reducing head weight and marketability. A small blemish on the head can still result in rejection or reduce its value, as the product is marketed in its fresh state and retailer protocols have stringent quality regulations to be met.

This study aims to evaluate the activity of new disease control programmes involving fungicides and biological control agents for control of the broad spectrum of pathogens that occur in lettuce crops. The best combinations of treatments for control of the various pathogens were investigated, whilst diversifying programmes to reduce the risks of both unacceptable residues at harvest and for selecting for fungicide resistant strains.

Project aim(s)

To carry out an evaluation of the broader efficacy of various approved and novel fungicides and bio-pesticides on both protected and outdoor lettuce in order to formulate a series of disease control programmes and strategies for the control of the most important pathogens of lettuce e.g. *Bremia lactucae, Botrytis cinerea, Rhizoctonia solani* and *Sclerotinia sclerotiorum* and any other incidental pathogens that happened to occur in the trial sites.

Project objective(s):

1. To conduct *in vitro* & *in vivo* (*in planta*) studies to screen new experimental products for the control of *Sclerotinia*, *Botrytis, Rhizoctonia & Bremia.* Select those most effective for *in vivo* screening in replicated field & glasshouse trials.
2. To carry out replicated trials in both field and glasshouse lettuce to a) evaluate the activity of the short-listed novel products against the primary pathogen targets and b) to

compare a range of integrated fungicide/bio-control programmes designed to investigate and optimise their broader efficacy and crop safety.

3. To validate the integrated programmes not only in terms of efficacy and crop safety but also with respect to residue levels through a series of multi-residue analyses at harvest to ensure retailer and consumer acceptance of the optimised programmes.
4. Prepare Annual & Final Reports, including HDC articles and an updated Factsheet to effectively communicate new knowledge to the industry

Each section of the report has been written according to the project objectives and includes, in order: *In vitro* screening, autumn 2012 Field Trial, spring 2013 Field Trial, autumn 2012 Protected Trial, spring 2013 Protected Trial.

Materials and methods

In vitro screening

A wide range of active ingredients were selected encompassing existing and new fungicide groups, e.g. Succinate Dehydrogenase Inhibitors (SDHIs) and other novel formulations as well as various biological products. Some are already approved for use on lettuce, whilst others were selected based on their activity against similar pathogens in other crops (Table 1).

Potato dextrose agar plates were prepared and amended with different concentrations of each chemical (ai) (0, 2, 20 and 100 ppm). Mycelial plugs (5 mm diameter) of each pathogen were placed on the plates for three days and the growth of the mycelium across the agar was measured. Percentage inhibition was calculated in relation to the growth of the mycelium across control untreated agar plates. *Botrytis*, *Rhizoctonia* and *Sclerotinia* (*S. sclerotiorum* and *S. minor*) were tested. As *Bremia* is an obligate biotroph oomycete it will not grow on agar plates, so *Phytophthora*, another oomycete which will grow on agar plates, was used to represent this pathogen. For the biological control organisms, the product was applied to the surface of the agar plate based on the recommended rate per hectare. For *Coniothyrium minitans,* which is active against sclerotia of *Sclerotinia*, a sclerotium was placed on the agar plate instead of a mycelial plug.

Table 1. Active ingredients tested *in vitro*.

Active ingredient	Example Product	Mode(s) of Action or Chemical Group(s)
HDC F148	Experimental	Succinate Dehydrogenase Inhibitor (SDHI)
HDC F149	Experimental	SDHI
HDC F152	Experimental	SDHI
HDC F155	Experimental	SDHI
HDC F156	Experimental	SDHI
HDC F157	Experimental	SDHI
boscalid	Filan	SDHI
boscalid/pyraclostrobin	Signum	SDHI/ Quinone outside Inhibitor (QoI)
pyraclostrobin	Vivid	QoI
azoxystrobin	Amistar	QoI
HDC F150	Experimental	QoI/ ethyl-phosphonate
cyprodinil	Unix	Anilino-Pyrimidine (AP)
pyrimethanil	Scala	AP
cyprodinil/fludioxonil	Switch	AP/ phenylpyrrole (PP)
tolclofosmethyl	Basilex	aromatic hydrocarbon
fluopicolide/propamocarb HCl	Infinito	benzamide/carbamate
HDC F146	Experimental	QoI/ Carboxylic Acid Amide (CAA)
dimethomorph	Paraat	CAA
dimethomorph/mancozeb	Invader	CAA/dithiocarbamate
mandipropamid	Revus	CAA
propamocarb HCl	Filex	carbamate
mancozeb	Dithane	dithiocarbamate
thiram	Thianosan	dithiocarbamate
iprodione	Rovral	dicarboxamate
prochloraz	Octave	Demethylation Inhibitor (DMI)
HDC F158	Experimental	DMI
fosetyl-aluminium	Aliette	ethyl-phosphonate
metalaxyl-M	Subdue	Phenyl Amide (PA)
metalaxyl-M/mancozeb	Fubol Gold	PA/dithiocarbamate
HDC F153	Experimental	Quinone x Inhibitor (QxI)/CAA
fenhexamid	Teldor	Sterol Biosynthesis (SBI)
HDC F145	Experimental	Plant Extract
Bacillus subtilis	Serenade	Biological - Bacterium
Coniothyrium minitans	Contans	Biological - Fungus
HDC F154	Experimental	Biological - Fungus

Autumn 2012 Field Trial

Aug/Sept 2012

Programme design

Programmes were designed taking into account requirements for the number of permitted applications, harvest intervals, diversification of different active ingredients and the spectrum of diseases expected. Typically fungicides are applied every 7–10 days commercially to maintain protection against downy mildew. All the fungicides used are protectants but some have known problems with pesticide residues in produce if applied too late in the programme, e.g. dithiocarbamates. Although these are very effective, and as multisite inhibitors useful as part of an anti-resistant strategy, they often have long harvest intervals to minimise the residue risk, therefore they are best used early in the programme to give good early protection with low risk. Subsequent further applications can then be made using different actives with action against downy mildew to maintain protection. It is important to vary the chemical groups used within the programme to guard against fungicide resistance, and where single actives are used that have a high resistance risk, they are best used in combination as a tank mix e.g. azoxystrobin. When tank mixes are used it is important to check that the conditions on each product label can be met when both products are mixed e.g. that rates, harvest intervals and conditions of application can be complied with.

It is also advisable to apply a fungicide with activity against *Botrytis cinerea* and *Sclerotinia* early in the treatment programme. This is because the highest risk of infection from these pathogens is from damage at planting as *Botrytis* is an opportunist secondary pathogen which will quickly colonise any damaged or wilting tissues. *Sclerotinia* is soil-borne and young leaves need to be protected against apothecial infection before the lettuce produces its head. As the lettuce matures it becomes more difficult to effectively target the older leaves which can act as a senescing substrate for germinating ascospores released from apothecia.

The programmes designed using the principles described above are shown in Table 2. There were additional programmes (e.g. treatment 4), which tested a biological product alone. Treatment 7 is strongly targeted to downy mildew control, treatment 12 which tests the activity of a single active ingredient, whilst treatment 16 integrates chemical and biological components.

Table 2. Fungicide programmes in Autumn Field Trial, Norfolk 2012.

	Fungicide treatments and rates			
Trt	**T1**	**T2**	**T3**	**T4**
	at planting	**7-10 days after T1**	**7-10 days after T2**	**7-10 days after T3**
	28 August	**3 September**	**13 September**	**26 September**
1	Untreated			
2	Amistar 1N + Karamate 1N	Signum 1N	Fubol Gold 1N	Revus 1N
3	Switch 1N + Karamate 1N	Amistar 1N	Fubol Gold 1N	Revus 1N
4	HDC F145 1N	HDC F145 1N	HDC F145 1N	HDC F145 1N
5	Switch 1N + Karamate 1N	Amistar 1N + Revus 1N	Fubol Gold 1N	HDC F145 1N
6	Amistar 1N + Karamate 1N	Previcur Energy 1N	Infinito 1N	HDC F145 1N
7	HDC F150 0.5N	Previcur Energy 1N	Fubol Gold 1N	Revus 1N
8	Amistar 1N + Karamate 1N	Previcur Energy 1N	Infinito 1N	Paraat 1N
9	Previcur Energy 1N	Previcur Energy 1N + HDC F151 1N	Infinito 1N	Revus 1N
10	HDC F146 1N	Invader 1N	HDC F146 1N	Paraat 1N
11	Signum 1N	Invader 1N	Infinito 1N	Paraat 1N
12	Paraat 1N	Paraat 1N	Paraat 1N	Paraat 1N
13	Amistar 1N + Karamate 1N	Signum 1N	Infinito 1N	Revus 1N
14	HDC F150 0.5N	Signum 1N	Infinito 1N	Paraat 1N
15	Amistar 1N + Karamate 1N	Signum 1N	Infinito 1N	HDC F145 1N
16	Signum 1N	Signum 1N + Serenade 0.8N	Serenade 0.8N	Serenade 0.8N
17	Untreated			

Trial design

The 17 treatment programmes above were set out in a randomised block design replicated four times to give a total of 68 plots. Double replication of the untreated plots was used to allow for a high degree of variance with the high number of plots and the size of the trial. Each plot consisted of a 5m length of bed to give at least 60 lettuces per plot, and a bed was 1.8 metres wide with four rows of lettuces. The trial was carried out using a commercial crop of iceberg lettuce of cultivar Excalibur, which is a variety susceptible to downy mildew. Pathogen infection was by natural occurrence, and the likelihood of infection was increased

by carrying out the trial in late summer and autumn when conditions are usually favourable for downy mildew.

Treatment applications

Treatments at all timings were applied with an Oxford precision knapsack sprayer with a 2m boom using a fine – medium spray at 2.5 bar pressure. The first 2 applications (timing 1 (28 August) and 2 (3 September) were applied in a water volume of 200 l/ha with 02F110 flat fan nozzles. This gives a fine quality spray for good coverage, in addition to good retention on leaves of a small target such as the small lettuce seedlings. The final 2 sprays (timing 3 (13 September) and 4 (26 September) were applied in water volume of 400l/ha with 04F110 nozzles to give a medium spray with good coverage while minimising drift.

Assessments

The plots were assessed at each spray timing and at harvest for incidence and severity of any of the target pathogens under investigation and crop safety. Disappointingly, only downy mildew was observed and this was scored by recording the percentage of leaf area affected using EPPO guidelines. A whole plot score was used when disease levels were low, and as disease progressed a smaller sample of the plot could be used as representative of the whole plot e.g. 10 or 20 heads of lettuce. A measure of yield was also taken at harvest of the weight of 10 trimmed heads of lettuce. Samples from each treatment were sent for residue analysis. Weather conditions were recorded at each spray timing. Typical symptoms of downy mildew in the experiment are shown in Figure 6.

Figure 6. (a) Early downy mildew lesion with sporulation on underside of leaf and typical chlorotic angular lesion. (b) Severe mildew infection with angular chlorotic and necrotic patches on lower leaf.

Spring 2013 Field Trial

March to June 2013

There were 15 treatments plus 2 untreated controls. Treatments were replicated four-fold on crisphead lettuce cv. Robinson. The treatment programmes are shown in Table 3.

Table 3. Fungicide programmes in Spring Field Trial, Stafford 2013

	Treatment timing			
Trt	**T1 2-4 days post planting 16 April**	**T2 7-10 days after T1 26 April**	**T3 7-10 days after T2 3 May**	**T4 7-10 days after T3 16 May**
1	Untreated	Untreated	Untreated	Untreated
2	Amistar 1N + Karamate 1N	Signum 1N	Fubol Gold 1N	Revus 1N
3	Switch 1N + Karamate 1N	Amistar 1N	Fubol Gold 1N	Revus 1N
4	HDC F145 0.5N + Revus 1N	HDC F145 0.5N + Revus 1N	HDC F145 0.5N + Revus 1N	HDC F145 0.5N + Revus 1N
5	HDC F145 1N + Amistar 1N	Signum 1N	Fubol Gold 1N	Revus 1N
6	Revus 1N + HDC F147 0.5N	Revus 1N + HDC F147 0.5N	Revus 1N + HDC F147 0.5N	Revus 1N
7	Amistar 1N + Karamate 1N	Previcur Energy 1N	Infinito 1N	HDC F145 0.5N + Revus 1N
8	HDC F150 0.5N	Previcur Energy 1N	Fubol Gold 1N	Revus 1N
9	Amistar 1N + Karamate 1N	Previcur Energy 1N	Infinito 1N	Revus 1N
10	Previcur Energy 1N	Previcur Energy 1N + HDC F151 1N	Infinito 1N	Revus 1N
11	HDC F146 1N	Invader 1N	HDC F146 1N	Revus 1N
12	Signum 1N	Invader 1N	Infinito 1N	Revus 1N
13	Amistar 1N + Karamate 1N	Signum 1N	Switch 1N + Infinito 1N	Revus 1N
14	HDC F150 0.5N	Signum 1N	Infinito 1N	Revus 1N
15	Amistar 1N + Karamate 1N	Signum 1N	Switch 1N + Infinito 1N	HDC F145 1N
16	Signum 1N	Signum 1N + Serenade 0.8N	Amistar 1N	Revus 1N
17	Untreated	Untreated	Untreated	Untreated

Trial design

The 17 treatment programmes above were set out in a randomised block design replicated four times to give a total of 68 plots. Double replication of the untreated plots was used to allow for a high degree of variance with the high number of plots and the size of the trial. The two control treatments were combined in the Genstat analysis of variance, therefore 16 treatments are shown in the results tables. Each plot consisted of a 5m length of bed to give at least 60 lettuces per plot, and a bed was 1.8 metres wide with four rows of lettuces. The trial was carried out using a commercial crop of iceberg lettuce of cultivar Robinson, planted on 10 April 2013. Pathogen infection was by natural occurrence, and the likelihood of infection was increased by using a field with a history of *Sclerotinia* spp. and crop covers during the early part of the season because of the cold spring.

Treatment applications

Treatments at all timings were applied with an Oxford precision knapsack sprayer with a 2m boom using a fine – medium spray at 2.5 bar pressure. The first 2 applications (timing 1 (16 April) and 2 (26 April) were applied in a water volume of 200 l/ha with 02F110 flat fan nozzles. This gives a fine quality spray for good coverage, in addition to good retention on leaves of a small target such as the small lettuce seedlings. The final 2 sprays (timing 3 (3 May) and 4 (16 May) were applied in water volume of 400l/ha with 04F110 nozzles to give a medium spray with good coverage while minimising drift.

Assessments

The plots were assessed at each spray timing and at harvest for incidence and severity of downy mildew, other diseases and crop safety. Severity was scored by leaf area affected using EPPO guidelines. A whole plot score was used when disease levels were low, and as disease progressed a smaller sample of the plot could be used as representative of the whole plot e.g. 20 heads of lettuce. A measure of yield was not taken at harvest as the marketable heads were cut accidently by the grower just before the final assessment. The outer leaves of the plants remained *in situ* and were still assessable and these enabled data for *Botrytis* and *Sclerotinia* to be collected. There was also enough leaf material left for residue analysis to be done. Low levels of lettuce big vein virus were noted at the site.

Small samples of 6 trimmed heads were collected from selected treatments at the final assessment on 19 June, stored in a cold store overnight and then taken for residue analyses on the following day to Scientific Analysis Laboratories Ltd, Cambridge. Duplicate samples of treatments 1, 2, 8, 13, 15 and 16 were provided for a general pesticide residue

screen. Duplicate samples of treatments 1, 2, 8 and 13 were analysed for dithiocarbamate residues.

Autumn Protected Trial

Oct-Dec 2012

Programme design

In this trial there were 12 treatment programmes at four application timings. The treatments included an untreated, an industry standard, four commercial programmes, four experimental programmes, a straight conventional experimental active and a straight biological experimental active (**Table 4**). The programmes were focused on providing broad spectrum control of the various pathogens.

Table 4. Fungicide programmes in Autumn Protected Trial, STC 2012

	Application Timing			
Treatment No.	**T1 2-3 days post-planting**	**T2 10-14 days after T1**	**T3 10-14 days after T2**	**T4 10-14 days after T3**
1	Untreated	Untreated	Untreated	Untreated
2 Standard commercial programme	Amistar 1N	Fubol Gold 1N	Teldor 1N	Revus 1N
Commercial programmes				
3	Fubol Gold 1N	Signum 1N	Switch 1N	Serenade 1N
4	Signum 1N	Paraat 1N	Octave 1N	Revus 1N
5	Paraat 1N	Revus 1N	Amistar 1N	HDC F154* 1N
6	Amistar 1N	Switch 1N	Signum 1N	Revus 1N
Experimental programmes				
7	Amistar 0.5N + Serenade 1N	Switch 0.5N + Paraat 0.5N	HDC F145 1N	Serenade 1N
8	Basilex 0.5N	Octave 0.5N + HDC F150 0.5N	Revus 0.5N + Switch 0.5N	HDC F145 1N
9	Signum 0.5N	Rovral 0.5N + Scala 0.5N	Octave 0.5N + Previcur Energy 0.5N	Serenade + HDC F145 1N
10	Amistar 0.5N + Previcur Energy 0.5N	Teldor 0.5N + Previcur Energy 0.5N	Signum 0.5N + Previcur Energy 0.5N	Serenade + HDC F145
Straight Experimental Active Ingredients (to determine spectrum of activity & crop safety)				

11	HDC F153 1N	HDC F153 1N	HDC F153 1N	HDC F153 1N
12	HDC F145 1N	HDC F145 1N	HDC F145 1N	HDC F145 1N

* HDC F154 is a biological product marketed as a plant strengthener, but in this trial was used as a fungicide and was codes as it is not approved for this type of use on lettuce. Commercially the product may be used as a plant strengthener without approval.

Trial design

The 12 treatment programmes above were set out in a randomised complete block design replicated four times to give a total of 48 plots. Each plot was 1 metre wide and 1.2 metres long and was planted with 42 lettuces, of which 20 were assessed. A romaine lettuce cv. Corsair was used, which is a variety susceptible to many isolates of downy mildew. To increase the chances of infection by the target pathogens, the trial was done in a glasshouse which had been used in the past for lettuce disease trials and it was known to have high levels of fungal pathogens, especially *Sclerotinia* and *Rhizoctonia*, already present in the soil. The likelihood of infection was increased by carrying out the trial in autumn when conditions are usually favourable for downy mildew. No inoculation was necessary as *Bremia lactucae* and *Botrytis cinerea* infected the crop naturally.

Treatment applications

Treatments at all timings were applied with an Oxford precision knapsack sprayer with a 2 m boom using a fine – medium spray at 2 bar pressure. All applications were applied at a water rate of 200 l/ha with 01F110 flat fan nozzles.

Assessments

The plots were assessed three times for incidence and severity of each disease and crop safety. Severity was scored per plant on a 0-3 scale where 0 = no disease, 1 = low disease level, 2 = moderate disease level and 3 = high disease level. Typical severe symptoms of each disease in the experiment are shown in Figure 7.

Figure 7. (a) Downy mildew infection on leaves. (b) *Botrytis* stem base infection which has caused the plant to collapse. (c) Typical mycelial webbing of *Rhizoctonia* between lower leaves of plant. (d) *Sclerotinia* stem base infection which has caused the plant to collapse. Black sclerotia have been produced on the stem base. They remain in the soil and produce apothecia (inset, not to scale) which release spores that infect the following crop.

Crop Diary

11.9.12	Romaine lettuce cv. Corsair planted
1.10.12	1st treatment application
11.10.12	2nd treatment application
22.10.12	3rd treatment application
8.11.12	1st disease assessment
12.11.12	4th treatment application
28.11.12	2nd disease assessment
13.12.12	3rd disease assessment

Spring 2013 Protected Trial

May to June 2013

Programme design

In this trial there were 12 treatment programmes including an untreated control replicated four times. Four post-planting application timings were planned, but only three could be made. Those applied are described in **Table 5**. It does not include the fourth post-planting

application which was not applied. The treatments included an untreated, an industry standard, three commercial programmes, three experimental commercial programmes and four experimental programmes. Many of the programmes included Amistar to control *Rhizoctonia* so that they could be compared to the use of Basilex pre-planting. The programmes in this trial were designed to see how late fungicide applications could be made before harvest. Currently the majority of commercial fungicide applications are made in the first three to four weeks after planting, potentially exposing the crop to disease infections later on which could make heads unmarketable. Growers are cautious of applying fungicides close to harvest because they do not wish to exceed maximum residue limits (MRLs). These programmes were designed to space out the number of applications to give better control of fungal pathogens from planting to harvest and, by using half rates and tank mixes, minimise residues at harvest. The crop matured faster than expected and the final treatment applications could not be applied. The crop had to be harvested before the minimum recommended harvest intervals had been reached for many of the products. This enabled data to be gathered on whether reducing application rates also reduced residues at harvest.

Table 5. Fungicide programmes in spring protected trial, STC 2013

	Application Timing			
Treatment Type	**Pre-planting**	**T1 2-3 days post-planting**	**T2 10-14 Days after T1**	**T3 10-14 Days after T2**
1. Control	No application	Untreated	Untreated	Untreated
2. Standard Programme	Basilex 1N	Fubol Gold 1N	Signum 1N	Revus 1N
Commercial Programmes				
3	Contans 1N	Amistar 1N	Fubol Gold 1N	Paraat 1N
4	Contans 1N	Paraat 1N	Amistar 1N	Switch 1N
Experimental Commercial Programmes				
5	HDC F154 1N	Amistar 1N	Paraat 1N	HDC F145 1N
6	No application	Amistar 0.5N + Fubol Gold 0.5N	Signum 0.5N + Switch 0.5N	Paraat 0.5N + Rovral 0.5N
7	No application	Amistar 0.5N + Fubol Gold 0.5N	No application	Signum 0.5N + Paraat 0.5N
8	No application	Rovral 0.5N + Amistar 0.5N	Paraat 0.5N + Signum 0.5N	Switch 0.5N + Revus 0.5N
Experimental Programmes				
9	No application	HDC F147 1N	1N Revus	HDC F152 1N

10	No application	HDC F150 0.5N + HDC F152 0.5N	HDC F145 1N	Paraat 0.5N + HDC F152 0.5N
11	No application	HDC F153 1N	HDC F146 1N	HDC F153 0.5N + HDC F146 0.5N
12	No application	HDC F148 1N	HDC F148 1N	HDC F149 0.5N + HDC F148 0.5N

Trial design

The 12 treatment programmes above were set out in a complete randomised block design replicated four times to give a total of 48 plots. Each plot was 1 metre wide and 1.2 metres long and was planted with 42 lettuces, of which 20 were assessed. The variety used was a butterhead lettuce of cultivar Tahamata. To increase the chances of infection by the target pathogens, the trial was done in a glasshouse which had been used in the past for lettuce disease trials and it was known to have high levels of fungal pathogens, especially *Sclerotinia*, already present in the soil. *Rhizoctonia* was artificially introduced by applying vermiculite inoculated with the fungus to the soil pre-planting. *Bremia lactucae* was artificially inoculated by applying a spore suspension to six plants per plot on two occasions during the trial. *Botrytis cinerea* infected naturally.

Treatment applications

Treatments at all timings were applied with an Oxford precision knapsack sprayer with a 2 m boom using a fine – medium spray at 2 bar pressure. All applications were applied at a water rate of 200 l/ha with 01F110 flat fan nozzles.

Assessments

The plots were assessed three times for incidence and severity of each disease and crop safety. Severity was scored per plant on a 0-3 scale where 0 = no disease, 1 = low disease level, 2 = moderate disease level and 3 = high disease level.

At harvest the untrimmed weight per head, trimmed weight per head and number of marketable heads/plot were recorded. Three heads from each plot were collected and pooled into one bag per treatment. As the crop was harvested on a Friday, the samples were frozen and analysed at a later date by two different laboratories in order to get as many residue data as possible from the wide range of products used. However, neither laboratory could analyse for mancozeb (fresh samples necessary) or fosetyl-aluminium (methodology not available).

Statistical Analyses

The data for the outdoor trials were analysed using Genstat statistical software package and the data for the indoor trials were analysed using ARM statistical software package.

Results and Discussion

In vitro tests

A range of approved and unapproved conventional fungicides and biological control products were tested using *in vitro* (agar plate) testing to investigate their potential efficacy in controlling mycelial growth of *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani* and a *Phytophthora* sp. (used to represent *Bremia lactucae*, which will not grow on agar). Each conventional fungicide product was tested at 4 concentrations: 0, 2, 20 and 100ppm of the active ingredient. Wherever possible products with single active ingredients were used rather than dual a.i. products, however this was not always possible. Biological control products were applied to the surface of the agar at the label rate prior to inoculation with the pathogen. The results have been calculated as the percentage of inhibition of mycelial growth compared to the untreated (0ppm) control (Figure 8, Figure 9, Figure , Figure & Figure).

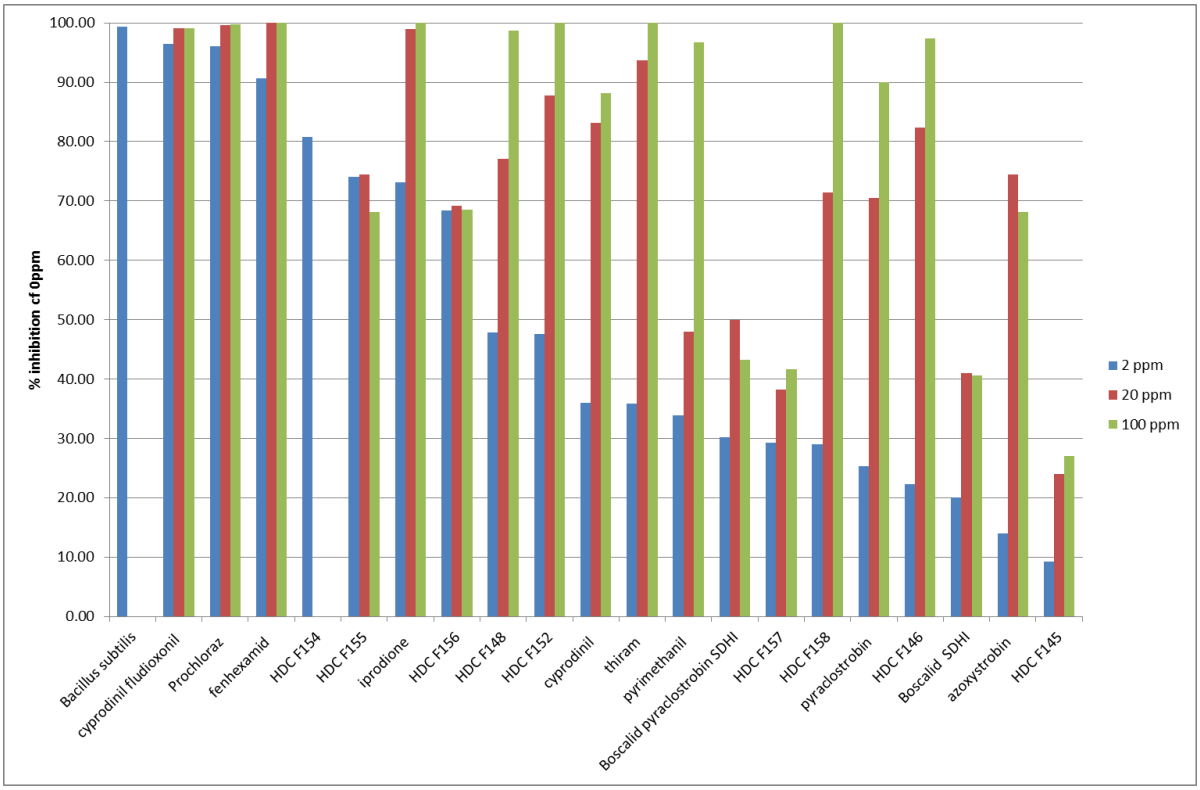

Figure 8. Percentage inhibition of mycelial growth of *Botrytis cinerea* on amended agar by a range of active ingredients at three different concentrations: 2ppm, 20ppm & 100ppm.

The results suggest that Serenade (*B. subtillis*), Switch (cyprodinil + fludioxonil), Octave (prochloraz) and Teldor (fenhexamid) are effective in this test even at the lowest concentration of ai. A range of other products have shown good efficacy at the highest concentration and this is encouraging.

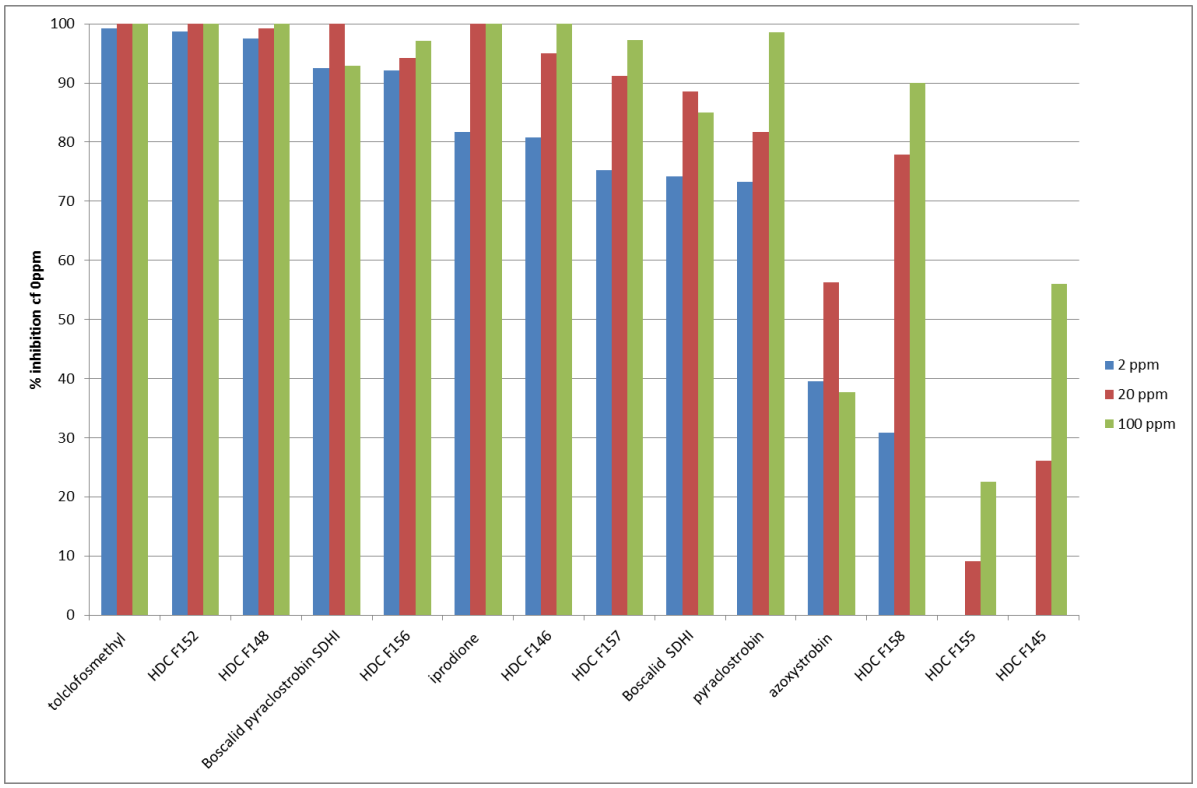

Figure 9. Inhibition of mycelial growth of *Rhizoctonia solani* on amended agar by a range of active ingredients at three different concentrations: 2ppm, 20ppm & 100ppm.

The inhibition of *R. solani* growth was >50% even at 2ppm of ai concentration with the majority of the products tested, and was excellent at the higher concentrations. We observed a rather disappointingly low level of inhibition in the test using Amistar (azoxystrobin), however it should be remembered that this test investigates only the inhibition of mycelial growth and in this respect is a limited test and such results must be interpreted with care. For example, many fungicide products have activity *in planta* e.g. the turning on of host defences, leaf greening and strengthening of plant cell walls which cannot be measured in this type of assay.

A high level of inhibition of mycelial growth was observed with the majority of the products tested against *S. sclerotiorum* and *S. minor*, particularly at the higher concentrations. It should be noted that this investigation compares the inhibition in mycelial growth, and therefore mirrors what might be expected in disease development and spread between plants, rather than inhibition of sclerotial germination.

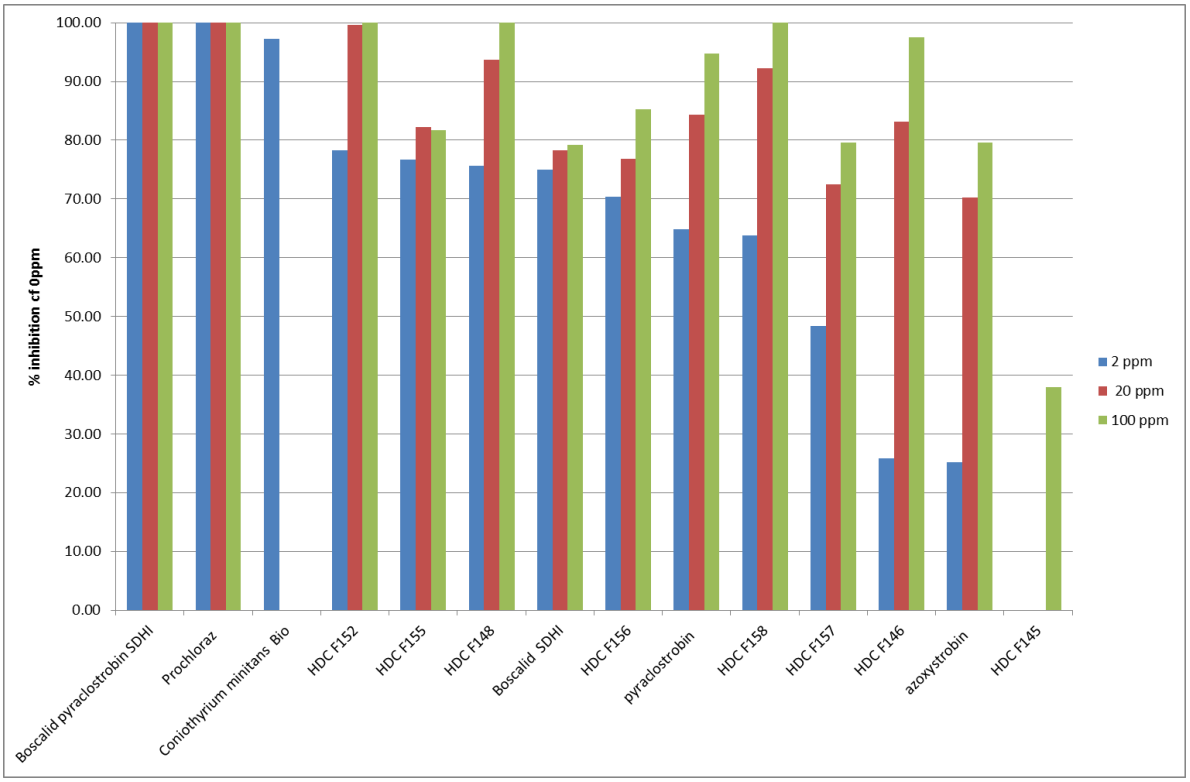

Figure 10. Inhibition of mycelial growth of *Sclerotinia sclerotiorum* on amended agar by a range of active ingredients at three different concentrations: 2ppm, 20ppm & 100ppm.

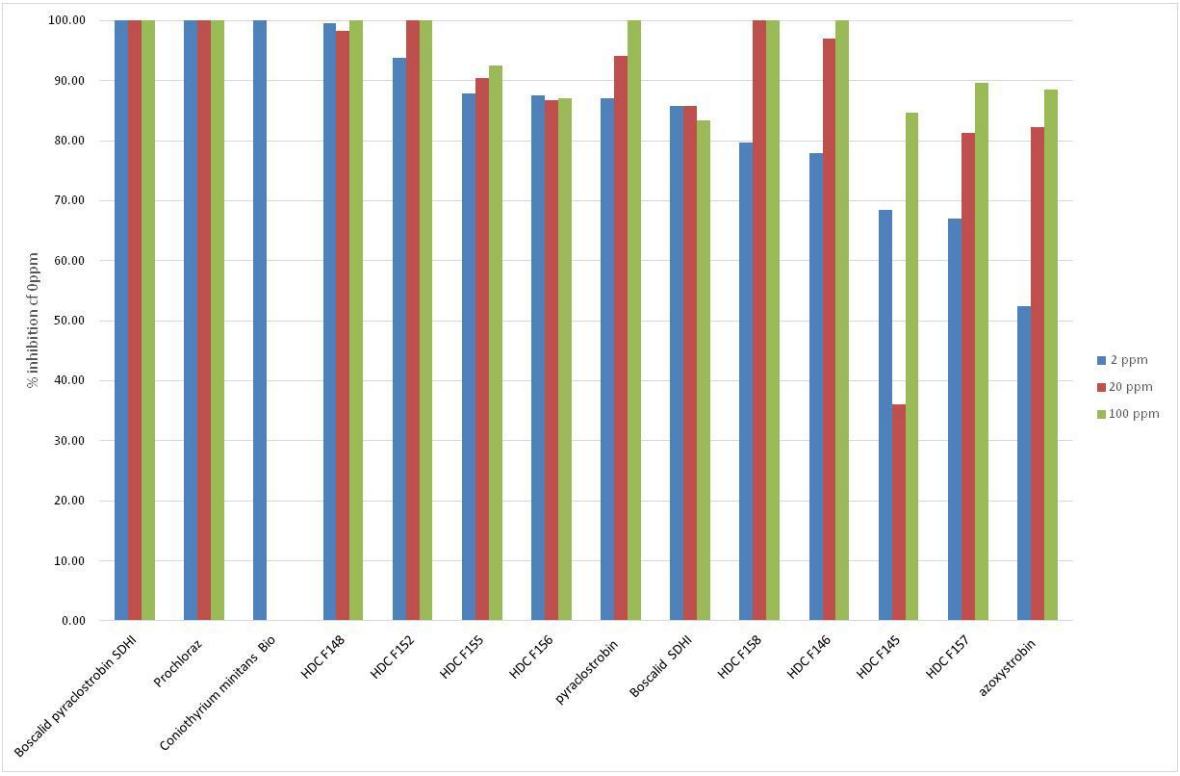

Figure 11. Inhibition of mycelial growth of *S. minor* on amended agar by a range of active ingredients at three different concentrations: 2ppm, 20ppm & 100ppm.

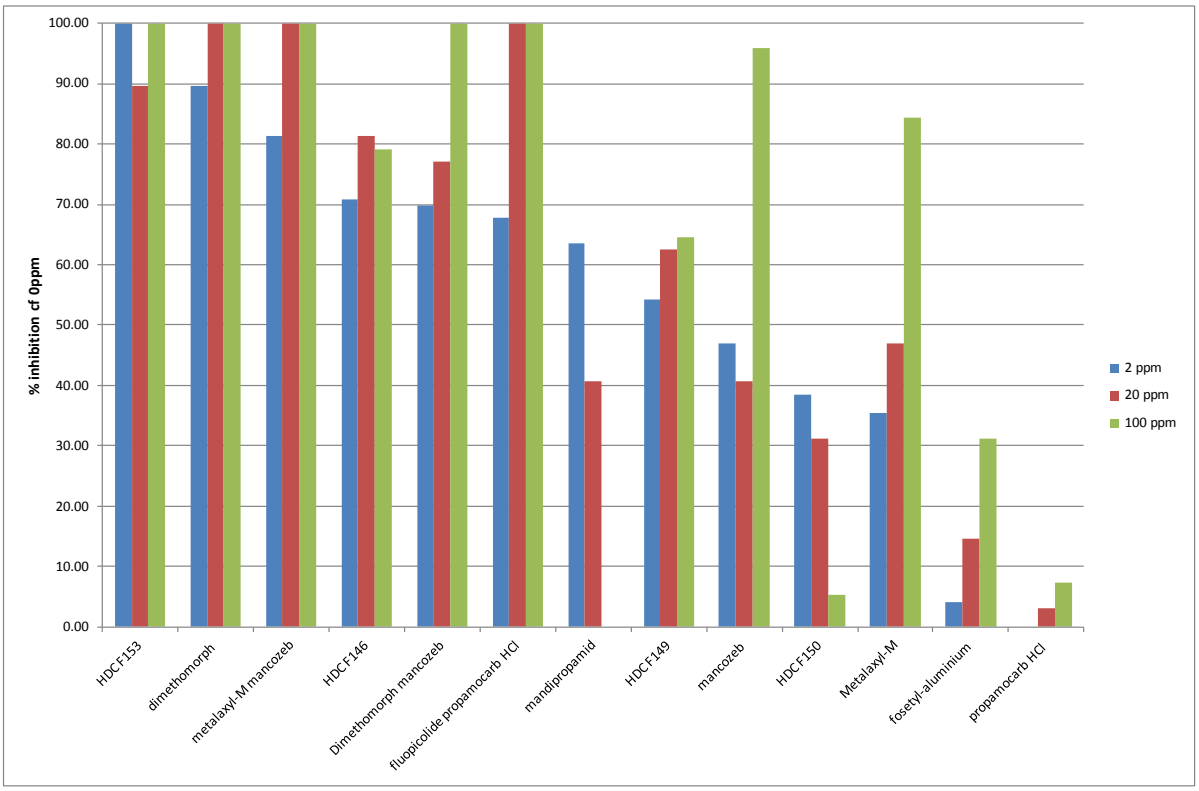

Figure 12. Inhibition of mycelial growth of *Phytophthora* sp. on amended agar by a range of active ingredients at three different concentrations: 2ppm, 20ppm & 100ppm.

A number of products showed some good activity against mycelial growth of *Phytophthora* including dimethomorph and mixtures containing mancozeb. Interestingly propamocarb HCl was rather ineffective in this study. Only low levels of inhibition were recorded with fosetyl-aluminium, however the mode of action of this product is known to involve the 'turning-on' of host defences and this activity cannot be measured in this type of assay.

The collected data were used to help devise spray programmes to target the main lettuce pathogens in the outdoor and protected lettuce studies which followed.

Autumn 2012 Field Trial

There was no downy mildew present at the first assessment when the first fungicide was applied at the start of the trial. Disease levels also remained very low at the second application, with only 2 plots showing 0.25 – 0.5% leaf area affected as an overall plot score. The weather was dry and warm with low relative humidity at the first application, and the crop was small and low in density which resulted in good air movement and reduced the chances of *Bremia* infection and spread. However, conditions at the later sprays (T3 and T4) became more favourable for disease spread, with temperatures of 19°C and moderate to high relative humidity which encouraged a moderate to high disease pressure in the crop and provided a stern test of the programmes. By 26th September downy mildew incidence in

the untreated plots had reached 66 – 78%, though the severity (% leaf area affected) was still relatively low (2%) at this point in the untreated plots. Favourable conditions for downy mildew continued between the final spray timing and harvest and downy mildew levels had escalated rapidly by the final assessment to 100% incidence in all plots and infection severity (% leaf area infected) in the worst affected treatments rose to 19.6%. Disease levels at each assessment are shown in **Table 6**. Treatments which are significantly better than the untreated are shown in bold in the table.

Table 6. Downy mildew severity (% leaf area affected) at each assessment and yield (kg/10 heads), Norfolk 2012

		severity (% leaf area affected)				
Trt	**Programme**	**Timing 2 3 Sept**	**Timing 3 13 Sept**	**Timing 4 26 Sept**	**Harvest 24 Oct**	**Yield (kg/10 heads)**
1	Untreated 1	0	0.34	2.18	11.32	2.52
2	Am+K/Sig/FG/Rev	0	0	**0.45**	**4.47**	2.15
3	Sw+K/Am/FG/Rev	0	0.05	**0.95**	**3.22**	2.35
4	F145 x 4	0	0	1.98	16.00	2.35
5	Sw+K/Am+Rev/FG/F145	0	0	**0.15**	**9.30**	2.13
6	Am+K/PrE/Inf/F145	0	0	**0.51**	15.22	2.75
7	F150/PrE/FG/Rev	0	0.13	**0.75**	**3.40**	2.77
8	Am+K/PrE/Inf/Par	0.50	0.13	**0.67**	9.60	2.53
9	PrE/PrE+F150/nf/Rev	0	0	1.23	**4.35**	2.25
10	F146/Inv/F146/Par	0	0	**0.28**	**7.92**	2.65
11	Sig/Inv/Inf/Par	0.25	0.50	**0.62**	**8.62**	2.23
12	Par x 4	0	0	**0.85**	11.75	2.50
13	Am+K/Sig/Inf/Rev	0	0.50	**0.26**	**7.52**	2.40
14	F150/Sig/Inf/Par	0	0	**0.75**	9.52	2.73
15	Am+K/Sig/Inf/F145	0	0.13	**0.67**	15.67	2.80
16	Sig/Sig+Ser/Sig/Sig	0	0	**0.66**	19.57	2.37
17	Untreated 2	0	0	1.62	14.70	2.73
F prob (significance)		NS	NS	0.006	<0.001	NS
LSD (48 d.f.)		-	-	1.034	5.182	-

Am = Amistar, K = Karamate Dry Flo Neotec, Sig = Signum, FG = Fubol Gold, Rev = Revus, Swi = Switch, F145 = HDC F145, PrE = Previcur Energy, Inf = Infinito, F150 = HDC F150, Par = Paraat, F151 = HDC F151, F146 = HDC F146, Inv = Invader, Ser = Serenade

Results of treatments giving significant control compared to the untreated plots are shown in bold.

NS = not significant.

The most effective programmes against downy mildew had Fubol Gold at T3 and/or Revus as the last treatment application (T4). This combination of chemicals maintained good control of disease severity despite moderate-high disease pressure and conditions conducive to downy mildew development at the end of the season. The availability of Revus provides opportunity to control the races of *Bremia lactucae* that are resistant to metalaxyl-M as was seen in HDC FV 357. Where resistance is known the combination of HDC F150, Infinito and Revus as the last two applications would provide a good alternative if approved. The top 4 combinations of products which kept mildew severity below 5% leaf area affected at harvest were:

1. Switch + Karamate Dry Flo Neotec, Amistar, Fubol Gold , Revus
2. HDC F150, Previcur Energy, Fubol Gold, Revus
3. Previcur Energy, Previcur Energy +HDC F150, Infinito, Revus
4. Amistar + Karamate Dry Flo Neotec, Signum, Fubol Gold, Revus

The downy mildew pressure increased exponentially between the last spray and harvest in the untreated and worst performing treatment combinations (Figure). This suggests that the last applied product for control of downy mildew needs to be very effective to maintain control to harvest, but also have a short harvest interval and minimal residue risk.

The most effective test programmes were only slightly better than the commercial programme. The period between the final treatment application and harvest was a week longer than scheduled due to adverse weather conditions. Arguably, downy mildew control was stretched in this experiment, though intermediate assessments also indicated that few treatments gave more than 75% control. At harvest most of the downy mildew was present on the outer leaves and there was very little disease on the heads. Thus downy mildew was removed by normal harvest trimming operations and there were no significant effects on yield (**Table 6**).

When designing programmes for disease control in lettuce crops it is important to consider other pathogens such as *Botrytis, Sclerotinia* and ringspot. Programmes that are effective for controlling downy mildew (e.g. HDC F150, Previcur Energy, Infinito and Revus) may not be as effective at controlling other non-oomycete diseases. Therefore it is good practice to integrate products with broad spectrum capabilities, and also consider which are the most likely pathogens to affect the crop and target applications accordingly. For example in warm, humid conditions where leaf wetness is high, *Sclerotinia* and *Botrytis* may also be significant problems.

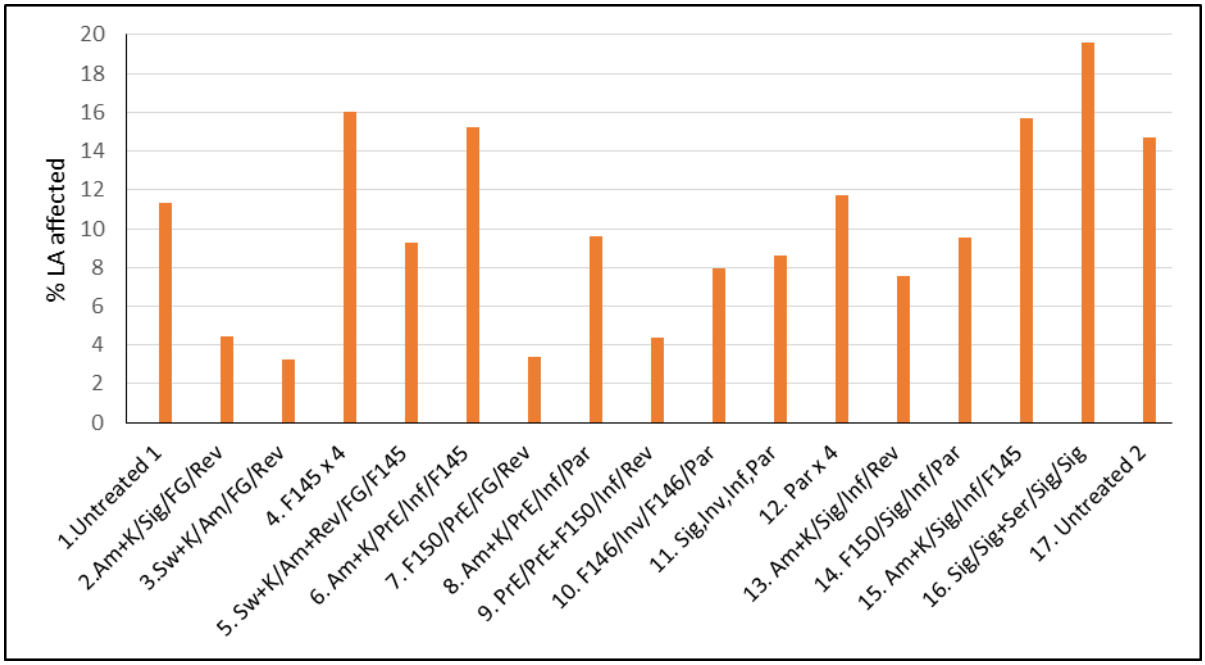

Figure 13. Chart showing the severity of downy mildew infection at harvest (24th Oct 2012) in the autumn field trial.

Spring 2013 Field Trial

The cold spring delayed crop development and growth was slow even under fine mesh covers. There was a history of *Sclerotinia* at this site but sclerotial germination was late in 2013 and activity only occurred from late May onwards. Low levels of severe *Botrytis* and *Sclerotinia* were evident on 10 June and the number of severely affected plants was counted. There was significantly more *Botrytis* in treatment 12 (Signum at T1) than in all the other treatments and treatment 16 (also Signum at T1) had more affected plants than the seven treatments with no *Botrytis* (Table 7). *Sclerotinia* was more prevalent in the untreated plots than in all the treated plots (significant $P<0.001$) but individual treatment differences were not significant. When *Botrytis* and *Sclerotinia* counts were combined, treatment 12 had more diseased plants than all the other treatments and the untreated control (Table 7). Six treatments had no plants with severe *Botrytis* and *Sclerotinia*.

Sclerotinia incidence was low at the final harvest (Table 8) but had affected the base of some plants and caused occasional head rots. *Sclerotinia* was most prevalent in the untreated (3.5% plants affected) and treatments 6, 9, 15 and 16. Disease levels were low and no treatment differences were significant.

Grey mould (*Botrytis cinerea*) was very common on the older leaves and sometimes caused a rot on the heads. *Botrytis* was present on 80-90% plants in most plots with rotting evident

in the petioles of several leaves. This was very close to causing losses from extra trimming of the heads. Moderately severe *Botrytis* (index of 2 or more) was present on 15% of untreated plants with no treatment giving significant control. Treatment 10 with HDC F151 at Timing 2 had a significantly lower incidence of *Botrytis* and a lower *Botrytis* severity than all the other treatments (Table 8). Only treatment 10 had significantly less severe *Botrytis* than the untreated.

Botrytis severity was higher in some treatments than in the untreated though not significantly so. This is not unexpected as fungicides aimed at downy mildew may affect microbial populations on the leaves and thereby reduce suppressive effects against other pathogens. It is important to quantify such effects so that targeting one disease does not aggravate others. This experiment suggests that good control of *Botrytis* may be difficult to achieve, though there may be scope to maintain protection by using suitable fungicides at T4. Products expected to give some control of *Botrytis* included Amistar, Signum and Switch. However, this late timing remains problematic because *Botrytis* develops around the base of the plant and may already be established on the oldest leaves. Achieving spray penetration to the base of the plant is likely to be difficult although systemic products would potentially help overcome this problem.

There was no downy mildew or ringspot recorded in this experiment.

No pesticide residues of significance were detected in any of the samples and all results remained below the limit of detection.

Table 7. *Botrytis* and *Sclerotinia* incidence, Stafford 10 June 2013

Trt	T1 2-4 days post-transplant	T2 7-10 days after T1	T3 7-10 days after T2	T4 7-10 days after T3	No. dead plants/plot *Botrytis*	No. dead plants/plot *Sclerotinia*	Total no. dead plants/plot
1	Untreated	Untreated	Untreated	Untreated	0.25	0.75	1.00
2	Amistar + Karamate	Signum	Fubol Gold	Revus	0.25	0.00	0.25
3	Switch + Karamate	Amistar	Fubol Gold	Revus	0.00	0.00	0.00
4	HDC F145 + Revus	HDC F145 + Revus	HDC F145 + Revus	HDC F145 + Revus	0.00	0.00	0.00
5	HDC F145 + Amistar	Signum	Fubol Gold	Revus	0.25	0.25	0.50
6	Revus + HDC F147	Revus + HDC F147	Revus + HDC F147	Revus	0.00	0.25	0.25
7	Amistar + Karamate	Previcur Energy	Infinito	HDC F145 + Revus	0.25	0.25	0.50
8	HDC F150	Previcur Energy	Fubol Gold	Revus	0.00	0.00	0.00
9	Amistar + Karamate	Previcur Energy	Infinito	Revus	0.25	0.00	0.25
10	Previcur Energy	Previcur Energy + HDC F151	Infinito	Revus	0.00	0.00	0.00
11	HDC F146	Invader	HDC F146	Revus	0.00	0.00	0.00
12	Signum	Invader	Infinito	Revus	**2.00**	0.25	2.25
13	Amistar + Karamate	Signum	Switch + Infinito	Revus	0.25	0.00	0.25
14	HDC F150	Signum	Infinito	Revus	0.50	0.00	0.50
15	Amistar + Karamate	Signum	Switch +Infinito	HDC F145	0.00	0.00	0.00
16	Signum	Signum + Serenade	Amistar	Revus	**0.75**	0.00	0.75
	Fpr				<0.001	NS (0.193)*	<0.001**
	SED				0.333	0.244	0.401
	LSD				0.670	0.491	0.806

*Significant P<0.001 for untreated v mean of all treatments

** Significant P=0.048 for untreated v mean of all treatments

Table 8. *Botrytis* incidence and severity and *Sclerotinia* incidence, Stafford 19 June 2013

Trt	T1 2-4 days post-transplant	T2 7-10 days after T1	T3 7-10 days after T2	T4 7-10 days after T3	*Botrytis* incidence % plants	Mean *Botrytis* severity 0-100	*Botrytis* incidence % plants (>index 2)	*Sclerotinia* incidence % plants
1	Untreated	Untreated	Untreated	Untreated	83	36.9	15.4	3.5
2	Amistar + Karamate	Signum	Fubol Gold	Revus	86	45.0	17.0	0.0
3	Switch + Karamate	Amistar	Fubol Gold	Revus	87	43.5	17.0	1.0
4	HDC F145 + Revus	HDC F145 + Revus	HDC F145 + Revus	HDC F145 + Revus	80	31.3	10.8	0.0
5	HDC F145 + Amistar	Signum	Fubol Gold	Revus	89	40.5	14.0	0.0
6	Revus + HDC F147	Revus + HDC F147	Revus + HDC F147	Revus	81	31.8	12.8	2.0
7	Amistar + Karamate	Previcur Energy	Infinito	HDC F145 + Revus	84	35.8	12.5	0.0
8	HDC F150	Previcur Energy	Fubol Gold	Revus	90	39.8	16.8	2.0
9	Amistar + Karamate	Previcur Energy	Infinito	Revus	80	36.0	16.3	3.0
10	Previcur Energy	P. Energy + HDC F151	Infinito	Revus	67	21.5	4.5	0.0
11	HDC F146	Invader	HDC F146	Revus	95	44.0	16.0	1.0
12	Signum	Invader	Infinito	Revus	90	43.3	16.8	0.0
13	Amistar + Karamate	Signum	Switch + Infinito	Revus	87	43.0	15.5	0.0
14	HDC F150	Signum	Infinito	Revus	87	38.0	12.8	0.0
15	Amistar + Karamate	Signum	Switch +Infinito	HDC F145	90	42.8	17.5	3.0
16	Signum	Signum + Serenade	Amistar	Revus	88	41.3	16.0	2.0
	Fpr	-	-	-	0.029	0.012	NS (0.101)	NS (0.711)
	SED	-	-	-	6.37	5.739	3.67	1.871
	LSD	-	-	-	12.81	11.538	7.379	3.762

Autumn 2012 Protected Trial

Treatments in this study included an untreated, an industry standard, four commercial programmes, four experimental programmes, a straight conventional experimental active and a straight biological experimental active.

Downy mildew and *Botrytis* infected the crop early and *Sclerotinia* developed at moderate to severe levels and therefore no artificial inoculations were required. However, and somewhat surprisingly, the levels of *Rhizoctonia* recorded were low, given the previous cropping with lettuce, absence of soil sterilisation and disease severity in earlier crops.

During the first assessment (8.11.12), downy mildew was present on approximately 30% of the untreated plants, but at a relatively low severity. It was recorded at around 50% incidence in T6 and T12 during this assessment and these two treatments also resulted in significantly higher severity of infection than the other treatments throughout the study. By the final assessment (13.12.12) T2 – the commercial standard programme showed the lowest incidence and severity of infection in the study (Figure 10).

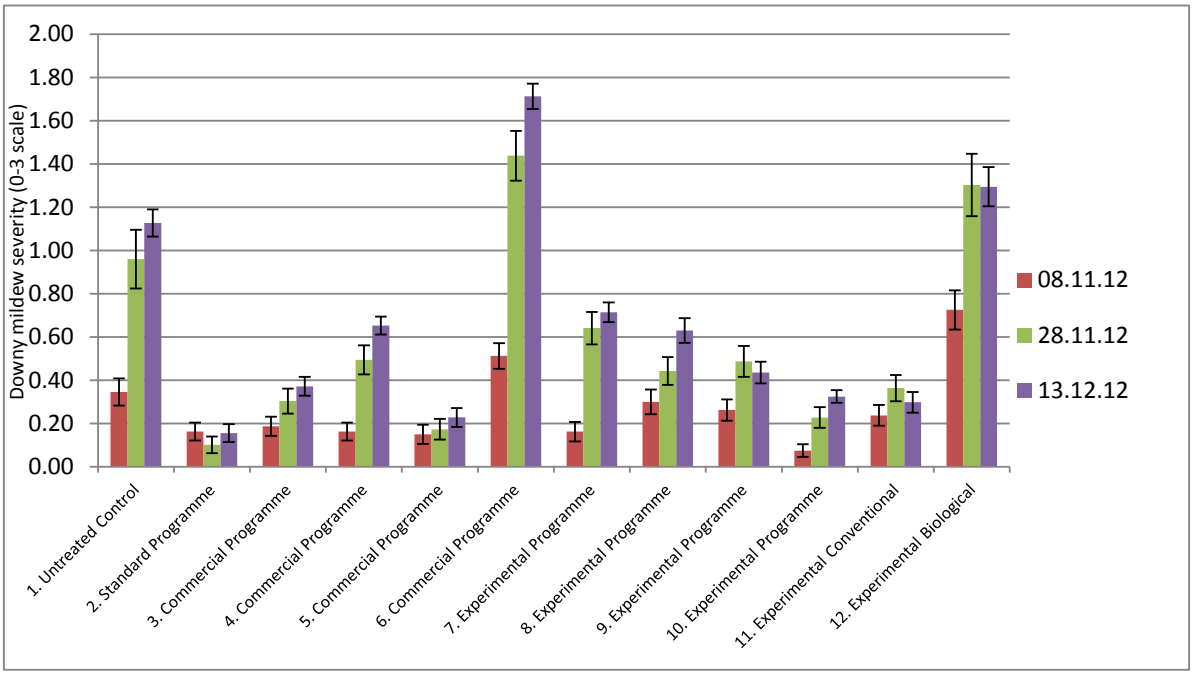

Figure 10. Chart showing the severity of Downy mildew infection in the autumn 2012 protected trial at STC (Error Bars represent ± Standard Error).

Botrytis and *Rhizoctonia* bottom rots were both recorded in this trial, but at low incidence and severity and no significant differences were observed between any of the treatments and the untreated plots.

Approximately 25% of the untreated plants were infected with *Sclerotinia sclerotiorum* at the first assessment date, although this was matched and exceeded by T11 and T5 respectively, where the incidence and severity of infection were significantly higher than in the other treated plots (Figure 11). At the first assessment a number of treatments reduced the incidence of infection (T3, 4, 7 and 10 in particular). Treatment 3 (FG/Sig/Sw/Ser) appeared to be the most effective programme for *Sclerotinia* control with only 3.75% incidence and 0.06 severity (0-3 index) at the end of the study. There was a good correlation between the number of dead plants and incidence of *Sclerotinia* and good correlations between incidences and severities for each disease in general. Full treatment results for each pathogen observed plus statistical analyses are shown in Appendix 4.

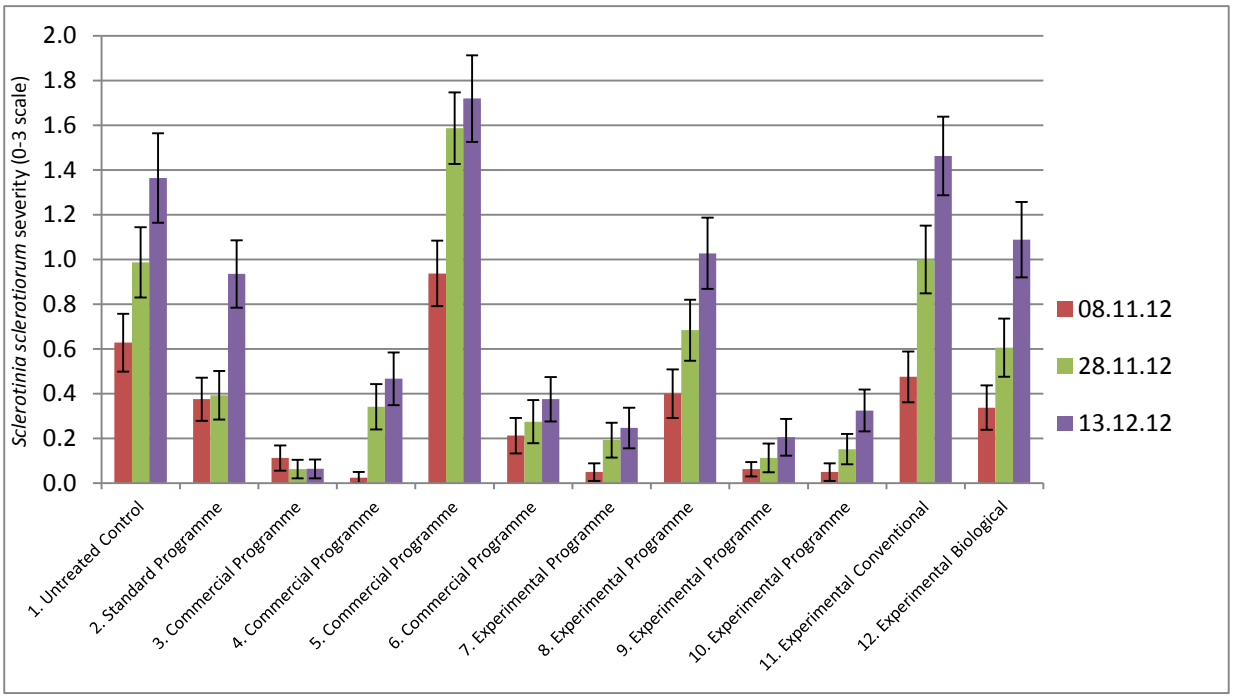

Figure 11. Chart showing the severity of *Sclerotinia sclerotiorum* infection in the autumn 2012 protected trial at STC (Error Bars represent ± Standard Error).

In terms of developing effective fungicide programmes to control such a broad range of target pathogens this initial trial has already demonstrated the challenges faced. For example, the treatments that performed best for control of downy mildew did not perform well against *Sclerotinia* or *Botrytis*. The treatments that performed best for control of *Sclerotinia* were relatively poor for downy mildew or *Botrytis* control and the treatments that were most effective against *Botrytis* were less effective against downy mildew or *Sclerotinia*. Therefore, in order to deliver a broad and effective treatment programme, it will be necessary to develop either tank mixes with different active ingredients (possibly at reduced

rates) to maintain broad spectrum protection throughout or to tailor the fungicide programme based on climatic factors and relative to disease risk.

In this first study, the standard programme provided significantly better control of downy mildew, but it performed poorly against *Botrytis* and below average against *Sclerotinia*. One of the commercial programmes and three of the experimental programmes performed reasonably well against all diseases. As disease levels, predominantly *Sclerotinia*, in the glasshouse were so high by the end of the trial most of the plants in each plot had died or were severely diseased, so there were insufficient heads to harvest or for samples to be taken for residue analyses.

Spring 2013 Protected Trial

Four post-planting application timings were planned, but only three could be made as the crop matured quickly.

No *Bremia lactucae* was observed in the trial. There were quite high levels of *Botrytis* and moderate levels of *Rhizoctonia* and *Sclerotinia*. In the first two assessments (10th and 17th June 2013) a moderate incidence of *Botrytis* was observed in the majority of the treatments, however, by the final assessment (21st June) the symptoms had been masked by the high levels of *Rhizoctonia* and *Sclerotinia* in many of the plots. No significant differences in the incidence or severity of *Botrytis* were observed. There were significant differences between the levels of *Rhizoctonia* and *Sclerotinia* at all assessments and these differences remained fairly consistent from one assessment to the next.

Several of the commercial programmes which utilised products already approved for use on protected lettuce provided a significantly better control of the incidence and severity of *Rhizoctonia* in this study (T2, 4, 5, 6, 7 and 8). The programmes utilising experimental products and also T3, did reduce the incidence and severity of the *Rhizoctonia* basal rots, but not significantly so (**Figure 12**).

S. sclerotiorum was observed at relatively low levels during the first disease assessment (10.6.13), with no significant differences between the treatments, although the highest incidence was observed in T9 (-/F147/Revus/F152). At this time no significant differences between treatments were observed. The disease incidence and severity increased steadily and by the final assessment on the 21.6.13 disease incidence was between 21 and 80% across the trial. Almost 50% of the untreated plants were affected, whilst >80% of the plants receiving T10 (-/F150+F152/F145/Paraat+F152) were dead or dying from *Sclerotinia*

bottom rot. The best level of control was observed with the standard programme (T2 – Basilex/Fubol Gold/Signum/Revus). Several of the remaining treatments showed a moderate incidence, but low severity of infection. Treatments 2 and 7 resulted in a significantly lower severity of infection than T10 (Figure 13).

A full set of treatment data and analyses for this trial is provided in Appendix 5.

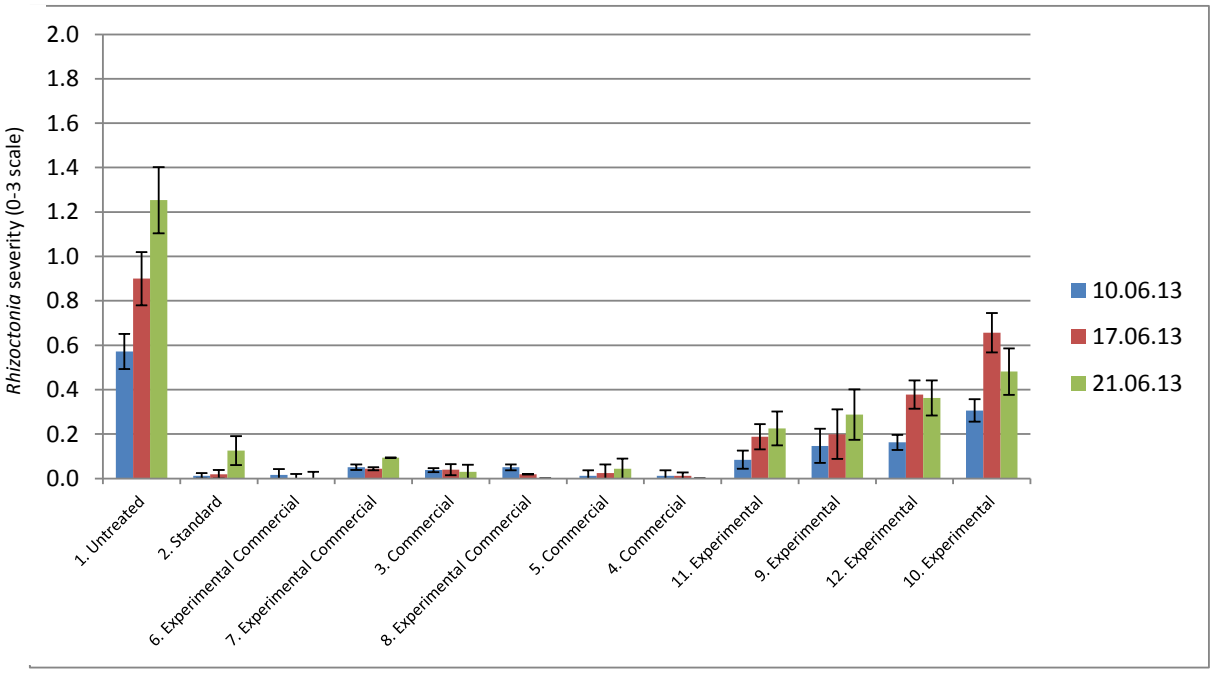

Figure 12. Chart showing the severity of *Rhizoctonia* infection in the spring 2013 protected trial at STC (Error Bars represent ± Standard Error).

Twenty plants/plot were harvested on the 21st June and the untrimmed and trimmed weight/plot were recorded (Figure 14).

Some low levels of pesticide residues were recorded at the end of the trial, but these were below the MRLs for all residues with the exception of HDC F152 which exceeded the MRL of 0.01 mg/kg when applied as a final application. The same residue level of 0.05 mg/kg was recorded for the application made at full rate as half rate. Considering the crop was cut before the minimum harvest interval, the policy of using half rates in tank mixes has meant that products could be applied closer to harvest if used at a lower rate.

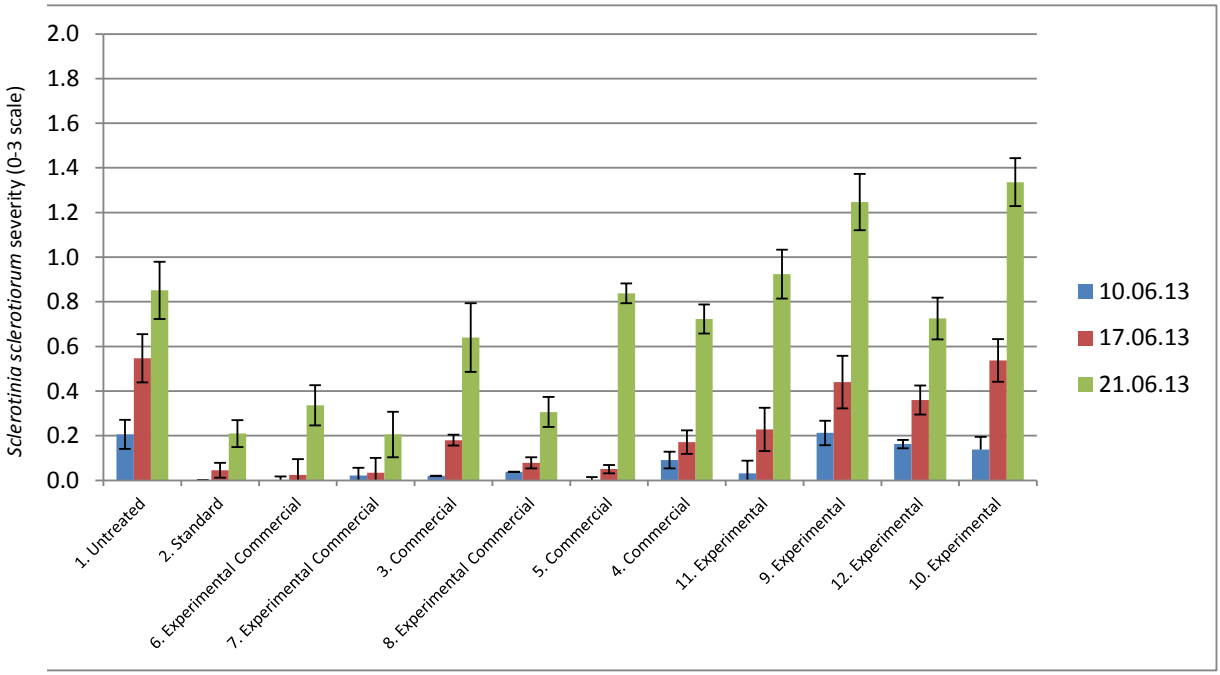

Figure 13. Chart showing the severity of *Sclerotinia sclerotiorum* infection in the spring 2013 protected trial at STC (Error Bars represent ± Standard Error).

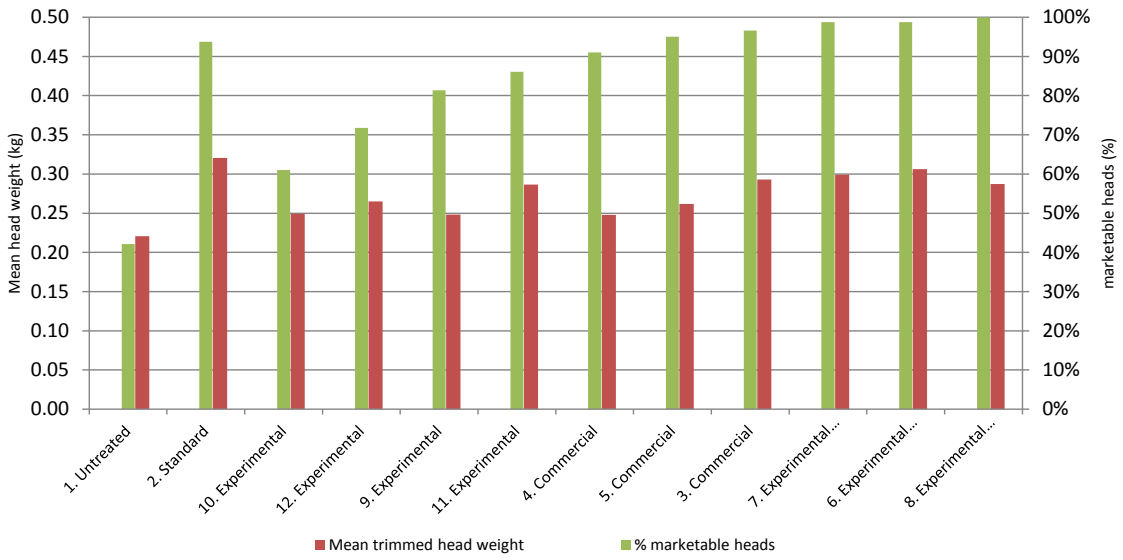

Figure 14. Mean trimmed head weight and percentage of marketable heads at harvest.

Treatment 3 (Commercial) – (Contans, Amistar, Fubol Gold, Paraat), treatment 6 (experimental commercial tank mixes) - (Amistar + Fubol Gold, Signum + Switch, Paraat + Rovral), and treatment 7 (experimental commercial tank mixes) - (Amistar + Fubol Gold, Signum + Paraat) had significantly fewer dead plants at the end of the trial than the industry standard. There were differences in the disease incidences and severities between these treatments and the standard, but these were not significant. Their mean head weight was

slightly below that of the standard, but not significantly lower. The percentage of marketable heads was significantly greater in these treatments than in the standard (Figure 14). All three programmes have three products in common: Amistar, Fubol Gold and Paraat. Interestingly in plate tests azoxystrobin, the active ingredient of Amistar, did not provide good inhibition of *Rhizoctonia* and *Sclerotinia*. Contans, which provided good inhibition of *Sclerotinia* in *in vitro* tests, may have helped control *Sclerotinia* in Treatment 3 and Signum, which provided good inhibition of *Rhizoctonia* and *Sclerotinia* in *in vitro* tests, may have helped to control these diseases in treatments 6 and 7, but it was not applied until later in the treatment programmes, as was Rovral in treatment 6, which does not explain why very low levels of these pathogens were recorded in earlier assessments. Treatment 7 only received two treatment applications in total, and yet was one of the best performing treatments. These results suggest that by using these products in the right tank mixes and applying at the right time, it should not be necessary to use Basilex as a pre-planting treatment. As no *Bremia* infected the trial it is not possible to evaluate the performance of Fubol Gold, although in the field trial it performed well at controlling the pathogen in treatment programmes that also included Amistar. Paraat was also used in the field trial programmes and provided quite good control of *Bremia*, although not as good as Fubol Gold.

None of the experimental programmes performed as well as the standard or any of the commercial programmes. Therefore these encouraging results mean that it may be possible to control these key diseases using existing approved products available to growers without the need to wait for new products to be registered and approved.

The project will use the knowledge acquired from the first year trials to devise more specific programmes to target these pathogens and refine the treatment applications.

Conclusions

In vitro tests

Moderate-good activity against oomycetes, *Botrytis*, *Rhizoctonia* and *Sclerotinia* spp. was identified in a number of active ingredients based on their ability to inhibit the mycelial growth *in vitro*. A number of the SDHI fungicides provided good to excellent inhibition of *Rhizoctonia* and *Sclerotinia*, though appeared less effective against *Botrytis* in this *in vitro* test. Some products, including some of the standard conventional products, inhibited *Botrytis* growth as well as *Rhizoctonia* and *Sclerotinia*. One experimental (coded) product inhibited all three pathogens, but on agar appeared to be most effective against *S. minor*.

Fungicides containing metalaxyl and dimethomorph provided good inhibition of the oomycete pathogen *Phytophthora* used as an alternate facultative organism to the obligate *Bremia* (lettuce downy mildew) which cannot be cultured *in vitro*. Fluopicolide & propamocarb hydrochloride (Infinito) also showed good activity against the target oomycete though currently this product is only approved for use on outdoor crops. Whilst metalaxyl was effective in the *in vitro* testing conducted here it is important to recognise that alternatives to the phenylamide fungicides are urgently required due to the prevalence of metalaxyl-resistant strains in the *Bremia* population.

Field Trials

Downy mildew is a major disease that affects UK lettuce crops and can be potentially devastating where epidemics occur. The disease is most likely to occur when conditions are cool (10 – 15^0C), humidity is high and when there is prolonged leaf wetness. Cultural controls such as choosing resistant varieties, plant spacing, row orientation, irrigation timing and weed control can all assist in reducing disease risk. In spring and autumn it is difficult at certain times to avoid these conditions and in these cases a robust fungicide programme is needed to complement and maintain varietal resistance and other disease control strategies. The project tested some experimental programmes including several products that may be approved in the near future. Four programmes tested stood out, these reduced % leaf area infection from 11 – 15% in the untreated to <5% leaf area infection and were:

1. Switch + Karamate Dry Flo Neotec, Amistar, Fubol Gold , Revus
2. HDC F150, Previcur Energy, Fubol Gold, Revus
3. Previcur Energy, Previcur Energy +HDC F150, Infinito, Revus
4. Amistar + Karamate Dry Flo Neotec, Signum, Fubol Gold, Revus

The availability of Revus provides opportunity to control the races of *Bremia lactucae* that are resistant to metalaxyl-M as was seen in HDC FV 357. Where resistance is known the combination of HDC F150, Infinito and Revus as the last two applications would also provide a good alternative subject to approval.

Therefore, there are promising options for the future as long as the residue tests can be satisfied.

Botrytis control was poor although in both trials the losses from disease were small. Only one treatment in the spring trial gave significant control of *Botrytis* and further work is required to identify the key factors for more effective control. There are concerns that fungicide resistant strains may be affecting the performance of fungicides particularly

strobilurins and SDHI products. Fungicide resistance tests may therefore be required to interpret product performance in future. However, *Botrytis* often occurs secondarily once downy mildew develops so by effectively controlling downy mildew, the risk from *Botrytis* via this route can be reduced.

Protected Trials

The autumn trial demonstrated that the development of cost-effective fungicide programmes to control a broad range of target pathogens will be a significant challenge. The treatments that performed most successfully against downy mildew did not perform particularly well against either *Sclerotinia* or *Botrytis*. The treatments that performed most effectively against *Sclerotinia* were relatively poor against *Bremia* or *Botrytis*. Surprisingly, the treatments that were most effective against *Botrytis* were not very effective against *Sclerotinia*. Tank mixtures and/or programmes of products will need to be adjusted according to the disease control spectrum required. Therefore, in order to deliver a broad and effective control programme, the approach of using reduced rate tank mixes using a range of different mode of action products, including biological products, has some merit both from an efficacy standpoint, but also from a residue and anti-resistance perspective.

Currently, many fungicides approved for use in protected lettuce require products to be applied early and usually within the first 2-4 weeks after planting to avoid MRL exceedance at harvest; the precise time often being dictated by the time of year the crop is being grown. Such restrictions on application timing potentially expose the crop to late infections close to maturity and risking economic loss once all the crop inputs have been made. Growers are naturally cautious of applying conventional fungicides closer to harvest because they cannot afford to risk exceeding the MRL (maximum residue level). Therefore one of the primary aims of the spring trial was to determine the scope for adjusting later application timings by either by using half rates and tank mixes or by incorporating biological pesticides for later applications to reduce any risk of fungicide residues at harvest. Unfortunately, in this trial, the crop matured faster than expected due to a change in the weather patterns and the final treatment applications could not be applied. As a result, many of the harvest intervals were not achieved but this did provide a good test for the novel programmes.

The data from these initial trials shows some early evidence to suggest that reduced (0.5N) rates of active ingredients (applied as various tank mixes) remained largely effective thus potentially providing an opportunity for broader disease control with a reduced risk of resistance development and/or pesticide residues at harvest.

There is, to date, little evidence from the trials to suggest that the biological products provided effective disease control in lettuce, especially where used alone. However, further exploration is still required to evaluate their use in integrated programmes to help reduce residue risk close to harvest and also reduce reliance on conventional fungicides thus further minimising the risk of resistance development whilst maintaining the health of the crop..

Knowledge and Technology Transfer

14/11/12 James Townsend attended British Leafy Salads Conference in Peterborough.

21/02/13 James Townsend attended British Leafy Salads Meeting and Residues Workshop at PGRO in Peterborough.

19/03/13 James Townsend attended British Leafy Salads Protected Lettuce Meeting at AHDB, Stoneleigh.

11/06/13 HDC Protected Edibles Meeting at STC with a tour of the site including a visit to the Protected Lettuce trial.

30/08/13 Article submitted to HDC News: 'Take guard on all fronts' published in October 2013 edition.

References

Gladders, P., Green, K., Huckle, A. and Kirkpatrick, L. (2010). Outdoor lettuce: evaluation of novel fungicides for downy mildew control. Horticultural Development Company Final report for project FV 357.

Horticultural Statistics (2013). Published by the Department for Environment, Food and Rural Affairs.

Appendices

Appendix 1. Weather conditions at spraying, Norfolk 2012

Target date (Timing)	Actual Date	Growth Stage	Weather (recorded at time of application)
Timing 1 4 days after planting	28/08/12	4 leaves	Temp: 27.2-28.7ºC RH: 44.1-46.5% Wind Speed: 2.7-3.1kph Sunny and 15% cloud cover Slight drift First 13 treatment applied with 04 nozzles for Amistar + Karamate treatments
Timing 2 7-10 after Timing 1 spray	03/09/12	4 Leaves	Temp: 18.9-19.4^{O}C RH: 87.4-89.1% Wind Speed 1.0-2.4 kph Cloudy and sunny Very slight drift
Timing 3 7-10 after Timing 2 spray	13/09/12	12 Leaves	Temp: 16.5-21.0^{O}C RH: 53.4-60.2% Wind Speed 1.8-2.4 kph Cloudy and sunny Very slight drift
Timing 4 7-10 after Timing 2 spray	26/09/12	GS 41	Temp: 16.0-21.4^{O}C RH: 62.4-84.7% Wind Speed 4.5-13.5 kph Cloudy and sunny – then drizzle 30 minutes after applications. No run off from plants. Very slight drift

Sprayer: OPS sprayer with 2m boom and 02 F110nozzles operated at 2 bars pressure and applying fungicides in 200 litres water/ha at timings 1 and 2 and F10/02 nozzles operated at 2 bars pressure applying treatments in 400 litres water/ha at timings 3 and 4.

Appendix 1. Weather conditions at spraying, field experiment 2, Stafford 2013

Target date (Timing)	Actual Date	Growth Stage	Weather (recorded at time of application)
Timing 1 4 days after planting	16/04/13	4 leaves	Temp:12.2-13.7 ^{O}C RH: 44.2-46.1% Wind Speed: 4.6-4.7 kph Fine, light wind Slight drift
Timing 2 7-10 days after Timing 1 spray	26/04/13	25% ground cover	Temp: 14.0-14.4^{O}C RH: 40.3-44.0% Wind Speed 3.3-3.6 kph Sunny and Dry Slight Drift
Timing 3 7-10 days after Timing 2 spray	03/05/13	5-6 leaves 50% ground cover	Temp: 14.8-17.1 ^{O}C RH: 51.6-56.7% Wind Speed: 3.0 kph Dry and Cloudy Slight Drift
Timing 3 7-10 days after Timing 3 spray	16/05/12	41-45 50% ground cover	Temp: 16.3-17.0ºC RH: 59-61% Wind Speed: 1.6-1.7 kph Warm and dry No Drift

Sprayer: OPS sprayer with 2m boom and 02 F110nozzles operated at 2 bars pressure and applying fungicides in 200 litres water/ha at timings 1 and 2 and F10/02 nozzles operated at 2 bars pressure applying treatments in 400 litres water/ha at timings 3 and 4.

Appendix 2. Spray timings STC, North Yorkshire Autumn 2012

Target date (Timing)	Actual Date	Growth Stage
T1: 2-3 days post-planting	01/10/12	7 true leaves
T2: 10-14 days after T1	11/10/12	10 true leaves
T3: 10-14 days after T2	22/10/12	12-14 true leaves
T4: 10-14 days after T3	12/11/12	Starting to heart up

Spray timings STC, North Yorkshire Spring 2013

Target date (Timing)	Actual Date	Growth Stage
Pre- planting	15/04/13	Pre-planting
T1: 2-3 days post-planting	13/05/13	3 days post-planting
T2: 10-14 days after T1	28/05/13	Starting to heart up
T3: 10-14 days after T2	11/06/13	Heads close to harvest

Appendix 3. Site details Field experiment 1, Norfolk 2012

Site:	Great Yarmouth, Norfolk
Field name/ GRef:	Whartons
Soil texture:	
Drainage:	Good
Soil analysis:	pH 6.5
(May 2010)	ADAS Indices – P 18.4 mg/l (2), K 122 mg/l (2-), Mg 60 mg /l (2)
	1.9 % organic matter
Crop: Carrots	Cultivar : Excalibur

	Planting date	:	
Harvest (farm)			24 October 2012
Harvest trial plots			24 October 2012

Site details Field experiment 2 Stafford 2013

Site:	Stafford		
Field name/ GRef:	Main road field		
Soil texture:	Sandy Loamy		
Drainage:	Good		
Soil analysis:	pH 6.5		
	ADAS Indices – P 75.6 mg/l (5), K 321 mg/l (3), Mg 82 mg /l (2)		
	1.3 % organic matter		
Crop: Carrots	Cultivar	:	Robinson
	Planting date	:	10 April 2013
Harvest (farm)			19 June 2013
Harvest trial plots			19 June 2013

Appendix 4 – Statistical analyses of STC protected crop 1 – Autumn 2012

Downy mildew incidence and severity in STC protected crop – autumn 2012

Treatment	Mean Downy mildew incidence (% plants/trt)			Mean Downy mildew severity (0-3 index)		
	8.11.12	28.11.12	13.12.12	8.11.12	28.11.12	13.12.12
1. Untreated	30.50ab	40.25a-d	46.00ab	0.34ab	0.99ab	1.11abc
2. Am/FG/Tel/Rev	16.25ab	9.00d	14.50b	0.16b	0.10c	0.16c
3. FG/Sig/Sw/Ser	18.75ab	28.00bcd	30.75b	0.19b	0.30bc	0.37c
4. Sig/Par/Oct/Rev	16.25ab	44.25a-d	46.00ab	0.16b	0.49bc	0.64bc
5. Par/Rev/Ami/F154	13.75ab	17.25cd	20.25b	0.15b	0.19bc	0.24c
6. Am/Sw/Sig/Rev	50.00ab	76.25a	80.00a	0.51ab	1.44a	1.71a
7. Am+Ser/Sw+Par/F145/Ser	15.00ab	53.50abc	54.00ab	0.16b	0.64bc	0.71bc
8. Bas/Oct+F150/Rev+Sw/F145	27.50ab	40.25a-d	46.50ab	0.30b	0.44bc	0.61bc
9. Sig/Rov+Sc/Oct+PE/Ser+F145	26.25ab	41.25a-d	30.00b	0.26b	0.49bc	0.44c
10. Am+PE/Tel+PE/Sig+PE/Ser+F145	7.50b	23.25bcd	31.50b	0.08b	0.23bc	0.33c
11. F153 x 4	23.75ab	34.00bcd	28.00b	0.24b	0.37bc	0.33c
12. F145 x 4	52.50a	61.00ab	53.75ab	0.73a	1.33a	1.35ab
LSD	25.4	25.7	28.6	0.3	0.5	0.6
SD	17.6	17.8	19.8	0.2	0.4	0.4

Means followed by the same letter do not differ significantly (Student-Newman-Kuels P=0.01

Botrytis incidence and severity in STC protected crop – autumn 2012

Treatment	Mean *Botrytis* incidence (% plants/trt)			Mean *Botrytis* severity (0-3 index)		
	8.11.12	28.11.12	13.12.12	8.11.12	28.11.12	13.12.12
1. Untreated	20.50a	30.25a	23.25a	0.43a	0.64a	0.58a
2. Am/FG/Tel/Rev	15.00a	26.00a	38.00a	0.23a	0.38a	0.69a
3. FG/Sig/Sw/Ser	18.75a	38.00a	34.25a	0.21a	0.42a	0.57a
4. Sig/Par/Oct/Rev	28.75a	30.75a	65.00a	0.39a	0.64a	1.17a
5. Par/Rev/Ami/F154	18.75a	23.00a	20.25a	0.26a	0.38a	0.33a
6. Am/Sw/Sig/Rev	13.75a	33.75a	31.25a	0.14a	0.34a	0.35a
7. Am+Ser/Sw+Par/F145/Ser	5.00a	32.00a	31.75a	0.13a	0.40a	0.38a
8. Bas/Oct+F150/Rev+Sw/F145	17.50a	11.50a	12.25a	0.21a	0.29a	0.31a
9. Sig/Rov+Sc/Oct+PE/Ser+F145	8.75a	15.00a	50.75a	0.09a	0.20a	0.69a
10. Am+PE/Tel+PE/Sig+PE/Ser+F145	17.50a	39.50a	66.00a	0.21a	0.57a	0.93a
11. F153 x 4	18.75a	21.25a	28.50a	0.25a	0.32a	0.50a
12. F145 x 4	30.00a	49.00a	34.00a	0.46a	0.90a	0.64a
LSD	22.4	36.9	43.4	0.3	0.5	0.6
SD	15.5	25.5	30.1	0.2	0.4	0.4

Means followed by the same letter do not differ significantly (Student-Newman-Kuels P=0.01

Sclerotinia sclerotiorum incidence and severity in STC protected crop – autumn 2012

Treatment	Mean *Sclerotinia* incidence (% plants/trt)			Mean *Sclerotinia* severity (0-3 index)		
	8.11.12	28.11.12	13.12.12	8.11.12	28.11.12	13.12.12
1. Untreated	25.75ab	37.75bc	47.50abc	0.64ab	0.99ab	1.38a
2. Am/FG/Tel/Rev	20.00ab	16.50bcd	37.25a-e	0.38b	0.39bc	0.96ab
3. FG/Sig/Sw/Ser	6.25b	3.75d	3.75e	0.11b	0.06c	0.06b
4. Sig/Par/Oct/Rev	1.25b	13.75bcd	19.25b-e	0.03b	0.34bc	0.45b
5. Par/Rev/Ami/F154	38.75a	61.75a	62.50a	0.94a	1.56a	1.80a
6. Am/Sw/Sig/Rev	10.00b	10.00cd	18.75b-e	0.21b	0.28bc	0.38b
7. Am+Ser/Sw+Par/F145/Ser	2.50b	8.75cd	9.00de	0.05b	0.19c	0.24b
8. Bas/Oct+F150/Rev+Sw/F145	16.25ab	25.50bcd	39.50a-d	0.40b	0.69bc	1.04ab
9. Sig/Rov+Sc/Oct+PE/Ser+F145	5.00b	3.75d	7.75de	0.06b	0.11c	0.21b
10. Am+PE/Tel+PE/Sig+PE/Ser+F145	2.50b	7.50cd	15.75cde	0.05b	0.15c	0.33b
11. F153 x 4	21.25ab	41.00b	52.25ab	0.48b	1.02ab	1.42a
12. F145 x 4	13.75b	25.00bcd	39.25a-d	0.34b	0.60bc	1.05ab
LSD	16.7	19.7	21.9	0.4	0.5	0.6
SD	11.6	13.7	15.2	0.3	0.4	0.4

Means followed by the same letter do not differ significantly (Student-Newman-Kuels P=0.01

Rhizoctonia incidence and severity in STC protected crop – autumn 2012

Treatment	Mean *Rhizoctonia* incidence (% plants/trt)			Mean *Rhizoctonia* severity (0-3 index)		
	8.11.12	28.11.12	13.12.12	8.11.12	28.11.12	13.12.12
1. Untreated	3.75a	0.00a	0.00a	0.06a	0.00a	0.00a
2. Am/FG/Tel/Rev	0.00a	2.50a	2.50a	0.00a	0.04a	0.03a
3. FG/Sig/Sw/Ser	1.25a	0.00a	0.00a	0.01a	0.00a	0.00a
4. Sig/Par/Oct/Rev	1.25a	0.00a	1.50a	0.03a	0.00a	0.01a
5. Par/Rev/Ami/F154	3.75a	1.50a	0.00a	0.10a	0.03a	0.00a
6. Am/Sw/Sig/Rev	2.50a	0.00a	3.75a	0.03a	0.00a	0.04a
7. Am+Ser/Sw+Par/F145/Ser	0.00a	0.00a	1.25a	0.00a	0.00a	0.01a
8. Bas/Oct+F150/Rev+Sw/F145	0.00a	0.00a	1.25a	0.00a	0.00a	0.03a
9. Sig/Rov+Sc/Oct+PE/Ser+F145	1.25a	0.00a	0.00a	0.04a	0.00a	0.00a
10. Am+PE/Tel+PE/Sig+PE/Ser+F145	1.25a	0.00a	1.25a	0.01a	0.00a	0.03a
11. F153 x 4	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
12. F145 x 4	3.75a	0.00a	0.00a	0.04a	0.00a	0.00a
LSD	4.4	1.7	4.4	0.1	0.0	0.0
SD	3.1	1.2	3.0	0.0	0.0	0.0

Means followed by the same letter do not differ significantly (Student-Newman-Kuels P=0.01

Appendix 5 – Statistical analyses of STC protected crop 2 – Spring 2013

Botrytis incidence and severity in STC protected crop – spring 2013

Treatment	Mean *Botrytis* incidence (% plants/trt)			Mean *Botrytis* severity (0-3 index)		
	10.6.13	17.6.13	21.6.13	10.6.13	17.6.13	21.6.13
1. Untreated	33.75a	45.98a	9.97a	0.32a	0.73a	0.17a
2. Bas/FG/Sig/Rev – Std prog	17.50a	50.00a	67.97a	0.11a	0.54a	0.58a
3. Con/Am/FG/Par	26.25a	38.27a	32.91a	0.20a	0.68a	0.35a
4. Con/Par/Am/Sw	46.25a	87.02a	56.53a	0.34a	0.82a	0.66a
5. F154/Am/Par/F145	53.75a	90.75a	35.77a	0.50a	0.47a	0.41a
6. -/Am+FG/Sig+Sw/Par+Rov	27.50a	87.94a	76.63a	0.22a	0.53a	0.68a
7. -/Am+FG/-/Sig+Par	3.75a	65.86a	57.33a	0.02a	0.69a	0.48a
8. -/Rov+Am/Par+Sig/Sw+Rev	26.25a	92.27a	87.77a	0.22a	0.62a	0.93a
9. -/F147/Rev/F152	32.50a	75.72a	11.78a	0.33a	0.41a	0.23a
10. -/F150+F152/F145/Par+F152	35.00a	52.56a	9.13a	0.27a	0.84a	0.12a
11. -/F153/F146/F153+F146	47.50a	85.18a	26.05a	0.40a	0.70a	0.23a
12. -/F148/F148/F148+F149	27.50a	75.14a	47.26a	0.21a	0.46a	0.56a
LSD	29.5	23.2	31.3	0.3	0.5	0.5
SD	20.5	16.0	21.7	0.2	0.3	0.4

First applications made as pre-planting trts.
Means followed by the same letter do not differ significantly (Student-Newman-Kuels P=0.01

Sclerotinia incidence and severity in STC protected crop – spring 2013

Treatment	Mean *Sclerotinia* incidence (% plants/trt)			Mean *Sclerotinia* severity (0-3 index)		
	10.6.13	17.6.13	21.6.13	10.6.13	17.6.13	21.6.13
1. Untreated	5.38a	33.26a	49.28a	0.21a	0.54a	0.85ab
2. Bas/FG/Sig/Rev – Std prog	0.00a	2.56bc	21.25a	0.00a	0.04a	0.21b
3. Con/Am/FG/Par	0.82a	13.87abc	56.97a	0.02a	0.26a	0.53ab
4. Con/Par/Am/Sw	6.07a	13.46abc	61.25a	0.09a	0.34a	0.72ab
5. F154/Am/Par/F145	0.00a	4.77abc	60.00a	0.00a	0.36a	0.84ab
6. -/Am+FG/Sig+Sw/Par+Rov	0.00a	0.75c	32.50a	0.00a	0.19a	0.34ab
7. -/Am+FG/-/Sig+Par	1.00a	5.18abc	35.00a	0.02a	0.19a	0.21b
8. -/Rov+Am/Par+Sig/Sw+Rev	0.57a	3.20bc	36.25a	0.04a	0.14a	0.31ab
9. -/F147/Rev/F152	14.87a	31.90a	80.00a	0.21a	0.04a	1.25ab
10. -/F150+F152/F145/Par+F152	3.58a	28.37ab	80.72a	0.14a	0.24a	1.33a
11. -/F153/F146/F153+F146	1.85a	14.95abc	77.50a	0.03a	0.11a	0.93ab
12. -/F148/F148/F148+F149	3.61a	14.62abc	51.25a	0.16a	0.25a	0.73ab
LSD	0.7	2.3	42.7	0.2	0.4	0.6
SD	0.5	1.6	29.6	0.1	0.3	0.4

Means followed by the same letter do not differ significantly (Student-Newman-Kuels P=0.01

Rhizoctonia incidence and severity in STC protected crop – spring 2013

Treatment	Mean *Rhizoctonia* incidence (% plants/trt)			Mean *Rhizoctonia* severity (0-3 index)		
	10.6.13	17.6.13	21.6.13	10.6.13	17.6.13	21.6.13
1. Untreated	42.73a	46.84a	49.26a	0.57a	0.49a	1.25a
2. Bas/FG/Sig/Rev – Std prog	0.57b	0.75bc	3.20b	0.01a	0.07a	0.13b
3. Con/Am/FG/Par	5.34ab	15.63abc	10.51ab	0.59a	0.14a	0.69ab
4. Con/Par/Am/Sw	0.57b	1.79bc	0.00b	0.01a	0.34a	0.00b
5. F154/Am/Par/F145	1.45b	0.75bc	1.79b	0.01a	0.13a	0.04b
6. -/Am+FG/Sig+Sw/Par+Rov	1.45b	0.00c	0.00b	0.02a	0.02a	0.00b
7. -/Am+FG/-/Sig+Par	1.14b	1.30bc	2.71b	0.05a	0.36a	0.09b
8. -/Rov+Am/Par+Sig/Sw+Rev	2.32b	1.30bc	0.00b	0.05a	0.31a	0.00b
9. -/F147/Rev/F152	3.37ab	19.25abc	12.27ab	0.15a	0.06a	0.29ab
10. -/F150+F152/F145/Par+F152	15.47ab	28.18ab	15.68ab	0.31a	0.08a	0.49ab
11. -/F153/F146/F153+F146	8.55ab	10.73abc	7.05ab	0.08a	0.78a	0.23ab
12. -/F148/F148/F148+F149	3.18ab	20.46abc	10.12ab	0.16a	0.38a	0.36ab
LSD	0.7	2.6	3.0	0.5	0.2	0.7
SD	0.5	1.8	2.1	0.4	0.1	0.5

Means followed by the same letter do not differ significantly (Student-Newman-Kuels P=0.01