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The results and conclusions in this report are based on a series of laboratory experiments and field trials on commercial crops of red beet. The conditions under which the experiments were carried out and the results generated have been reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are to be used as the basis for commercial product recommendations.

It should also be noted that many of the products tested in this work are experimental in nature and under no circumstances should they be used commercially. If anyone is in doubt regarding the current approval status of a particular product they should either, consult the manufacturer, check the status on an approved pesticide database or take independent advice from a BASIS qualified adviser.

AUTHENTICATION

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

Signature……

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FV 226c : GROWER SUMMARY

Red Beet : Further Elucidation of the Cause, Epidemiology and Control of Root Malformation Disorder (RMD)

Headlines

- Levels of root malformation disorder (RMD) in commercial red beet crops during 2004 were low; the majority of growers in the Isle of Axholme area experienced less than 1-2% of distorted roots at harvest. Occasional crops in the same region had up to 5% root malformation disorder. Elsewhere in the UK, the level of root distortion was also reported to be exceptionally low.

- RMD, low in 2004 crops, especially in the Isle of Axholme appeared to correlate fairly closely with the overall low incidence of downy mildew. The relatively warm, dry spring period was not conducive to infection by *P. farinosa*. The extensive use of Wakil treated seed and foliar applied fungicides, especially in high risk crops, is also likely to have had a significant impact on the incidence of the disorder during 2004.

- *Rhizoctonia* and *Pythium* spp. were not evident in trial or other commercial crops during routine monitoring over the season. However, *Aphanomyces cochlioides* caused a significant level of damping-off in the trial site at Westwoodside and, due to the patchy emergence and poor survival of seedlings, required a change of location at one of the four trial sites.

- At the Westwoodside site there was a significant correlation between the total number of distorted roots in each treatment and the application of d. mildew fungicides. Compared to the untreated control, all the oomycete fungicides applied significantly reduced the incidence of RMD. Unfortunately, symptom severity was only mild and caution is required with respect to any conclusions drawn from this study. The strong correlation between crop vigour at the end of the season and the applied fungicides treatments were attributed to the control of foliar disease, primarily *Cercospora* leaf spot with Amistar was the most effective product. The dithiocarbamate (mancozeb) component of Fubol Gold and Invader also proved surprisingly effective.

- At a separate commercial site in the Isle of Axholme area a moderate-high level of d. mildew was observed in the crop in early June 2004. Plants showing a variety of symptoms were ‘tagged’ to allow monitoring of the development of RMD symptoms. Plant samples were tested for the presence of DNA of d. mildew in the root tissues. Results from this tagging, monitoring and PCR testing provided further strong evidence to support to the hypothesis that RMD is caused by an internal or systemic infection by the d. mildew fungus *P. farinosa*.

- In parallel with this trials work efforts continue, in discussion with the manufacturers, HDC and other stakeholders to secure alternative fungicides for the control of d. mildew and to minimise the resistance risk from over reliance on single mode of action (and site specific) fungicides. In many respects, the key to accessing the wider array of the ‘blight’ fungicides is approval of the dithiocarbamate fungicide mancozeb as this is the preferred ‘protectant’ partner in many formulated blight products.

Background and Expected Deliverables

During early Autumn 1998 concerns were raised by a number of growers regarding the occurrence of an apparent new disorder or disease of red beet. As crops neared maturity roots were observed to be severely distorted (Plate 1).

Plate 1 : RMD affected beet in the field (right). Note proximity to adjacent healthy beet (left).

In addition to the distortion, affected roots had an elongated neck and, in some cases, had a thickened tap root. One particular characteristic of the affected beet was a russetting or corkiness around the shoulder of affected plants (Plate 2).

Plate 2 : Distorted roots of red beet with an elongated neck, russetting and corkiness around the shoulder.

The smaller or 'baby beet' size grades were reported to be particularly badly affected. The syndrome was referred to as root malformation disorder or RMD. Various estimates put economic losses due to RMD at around £1M/annum.

HDC sponsored a 2-year investigation at Stockbridge House during the period 1999-2001. Studies commenced on a broad basis in Year 1 to conduct a literature search, distribute a questionnaire to growers, conduct a series of pot studies and to eliminate a number of possible factors that could potentially have led to such severe root distortion. During this initial investigation, tests for 'Rhizomania' and other virus diseases were conducted, as were tests for herbicide injury, nematode infestation and bacterial pathogens. All tests proved negative.

In the second year of the project information gleaned from pot studies were used to design and undertake a series of replicated field-scale trials on commercial farms ~~to eva~~luate the performance of various experimental fungicides applied as seed treatments and post-emergent HV sprays. Individual sites responded moderately well to fungicides and at site 2 (Westwoodside) RMD symptoms were well controlled with metalaxyl-M applied as SL567 (for oomycete control) either as a seed treatment or drench application. At the other 2 sites levels of RMD were much lower. Some response from the applied products, particularly SL567A, Monceren (for *R. solani* control) and Biomex (also targeting *R. solani* primarily) was achieved. Based on the 2-year study, it was concluded that the most probable cause for RMD was a *Pythium-Rhizoctonia* complex, infection occurring at the seedling stage with the distortion symptoms developing as the roots enlarged. A recommendation was therefore made to pursue On- or Off-Label authorisation for the fungicide metalaxyl-M (SL567) and possibly azoxystrobin (Amistar).

In October 2002 growers, particularly in the Isle of Axholme region of South Yorkshire, again reported an extremely high incidence of RMD. On this occasion, it appeared that the problem developed quite late in the season (August-September). In some cases it was severe in fields that had not grown commercial crops in the Chenopodiaceae for several years or on land that had been down to grass for 20 years. As previously, the problem appeared to correlate closely with wet weather, in this case heavy rainfall during August after a prolonged dry spell. The reported absence of early symptoms and the presence of severe RMD in 'virgin' sites, rather than pointing to a soil-borne pathogen, tended to suggest aerial dissemination eg an aphid vectored virus or an air-borne fungus.

Plate 3 : Crown infection of red beet with downy mildew (*Peronospora farinosa* f.sp. *betae*).

Close inspection of affected crops noted a fairly heavy infestation of downy mildew caused by *Peronospora farinosa* f. sp. *betae* (Plate 3), a pathogen not noted at particularly significant levels in previous years.

As an oomycete this obligate pathogen could also be expected to be well controlled (subject to the absence of resistant strains in the pathogen population) by SL567A. In other crops downy mildew fungi eg *Peronospora viciae* in peas are reported to infect seedlings systemically to cause distortion, without obvious sporulation. However, reports of root distortion caused by d. mildew infection have not been generally reported. A web-based report from Oregon in the USA described root distortion symptoms in red beet (Plate 4) and attributed this to infection by the d. mildew fungus *P. farinosa*. The description of symptoms reported in Oregon correlated closely with those of RMD.

Plate 4 : Distorted roots of red beet, claimed to be caused by the downy mildew pathogen (Oregon, USA).

The HDC funded work in 2003 (FV226a) aimed to further investigate the role played by both soil- and air-borne pathogens in the RMD problem in a series of field-scale trials as a means of elucidating the primary cause. The primary objective/deliverable was to evaluate a soil sterilisation treatment in conjunction with a range of existing and novel fungicides. In addition, a literature search was undertaken which indicated that *P. farinosa* f.sp. *betae* could not infect wild *Chenopodiaceae*, therefore weed species, in or around commercial beet crops, were not acting as a reservoir for the disease. However, the same pathovar was also pathogenic on sugar beet and it was concluded that this crop could be a significant factor in pathogen survival and carry-over between red beet crops.

Towards the end of the project in 2003-2004 CSL, using samples provided by STC, developed a novel molecular method for DNA analysis of affected and unaffected roots. The reason for this was to develop a novel method against this obligate (non-culturable) pathogen to determine conclusively whether the downy mildew pathogen was implicated in, and responsible for, RMD in red beet. Initial indications from this work were very promising and an excellent correlation between root distortion and the presence of DNA of *P. farinosa* in the root tissues was gained.

New projects were therefore commissioned in 2004 to gain further evidence of efficacy and optimum timing of oomycete specific fungicides and to further validate the observed correlation between RMD symptoms and presence of *P. farinosa* DNA in affected roots.

Summary of the 2004 Project and Main Conclusions

(i) Fungicide Performance (Efficacy)

Following discussion with industry representatives two sites for trial purposes were identified on commercial farms in South Yorkshire. At each site the majority of the field was sown with Wakil XL treated seed, however approximately 12 beds were sown with the same variety of untreated seed to allow investigation of the role that Wakil XL might play in preventing a very early seedling infection with d. mildew. Red Beet seed cultivar Pablo was drilled at Site 1 (Westwoodside) on 24 May, and at Site 2 (West Butterwick) on 11 May. Fungicide applications commenced in the first week of June at both sites using Oxford Precision knapsack spray equipment at 14-day intervals.

Trial Site 1 (Westwoodside)

At this site seedling establishment was very poor, especially in large areas of the field. At the cotyledon stage leaf discoloration (reddening/purpling) was observed across the trial area and close inspection showed evidence of hypocotyl discoloration (blackening) and seedling collapse. Samples of affected seedlings were returned to the laboratory for detailed examination. Black-leg caused by the soil-borne fungus *Aphanomyces cochlioides* was confirmed on the affected seedlings and was considered to be the primary cause for the establishment problems at this site. The Wakil seed treatment appeared to have no effect on the *A. cochlioides* infection.

As only one fungicide application had been made to the trial area, it was agreed after consultation with the grower, to relocate the trial to a nearby field where cv. Crimson Globe had been sown on the 25 May. This field contained all Wakil XL treated seed, therefore it was not possible to include an untreated seed treatment in the new trial design.

Regular monitoring of the crop, prior to each fungicide application, revealed only a very negligible level of infection of *P. farinosa* during the trial period. Detailed grab sampling, and in-crop assessments showed the crop to be healthy, with little indication of the development of RMD in the beet.

The area was harvested in early November and by this stage low levels of RMD were found in the untreated control plots. However, even lower levels of root distortion were seen in each of the fungicide treatments. Whilst the distortion symptoms were only mild it provides further evidence for a link between infection by oomycete fungi and RMD. As seen during the work carried out in 2003, marked differences in plot vigour were noted between the fungicide treatments and this was again attributed largely to a *Cercospora* leaf spot infection.

Trial Site 2 (West Butterwick)

The site location at West Butterwick had not had red beet grown on it for several years. The crop at this site was very fast growing and healthy, with no leaf diseases present. No downy mildew developed at this site. Grab sampling carried out in late July showed no differences in plot vigour and no roots affected with RMD. By mid-late August the grower advised us that the crop was mature and the remainder of the field was to be harvested. As there was no likelihood of getting distortion developing in the roots at this late stage it was decided to terminate the trial at this stage.

(ii) Fungicide timing

An investigation into the timing of fungicide applications to control infection by *P. farinosa* was carried out at 2 sites during 2004. The trials were located close to the fungicide trials, but in areas of the field that had been drilled with non-Wakil treated seed. The cultivar Pablo was drilled at Westwoodside on the 24 May and at West Butterwick on the 11 May. A tank mix of Fubol gold (metalaxyl-M + mancozeb) and Invader (dimethomorph + mancozeb) was applied at 14-day intervals using an Oxford Precision knapsack sprayer. Treatments consisted of a series of different application timings with either 2 or 4 spray programmes. Treatment application commenced in early June with a maximum of 8 applications at Westwoodside and 6 at West Butterwick.

Careful monitoring of the trial areas prior to each fungicide application was carried out. No evidence of d. mildew or root distortion was seen at either site. The West Butterwick site was terminated relatively early in late August due to early maturity of the crop. The Westwoodside site was harvested on the 27 October. Negligible and insignificant levels of downy mildew, other leaf and root pathogens and RMD were recorded at harvest.

(iii) Plant 'Tagging' in 2004

During the first week in June our attention was brought to a commercial grower's field in the Isle of Axholme area. The grower reported the presence of d. mildew on the crop and was about to apply a fungicide to control the problem. After a site visit and discussion with the grower it was agreed that a 12m x 12m area in the centre of the field would be left unsprayed to allow us to monitor the development of the disease and any resulting RMD that might occur. A large number of plants, showing a range of different symptoms (see below) were tagged for monitoring throughout the season and arrangements were made to include a number of the tagged plants in the molecular testing carried out by CSL.

At the first site visit it was agreed to tag plants as follows:-

Category 1 : Healthy plants, no downy mildew or other symptoms visible
Category 2 : Early crown infection with downy mildew, sporulation clearly evident
Category 3 : Evidence of leaf distortion and multiple crown effect (some showing brown petiole symptom),
but no sporulation of downy mildew

The plants in the 3 categories above were identified and tagged on 14 June. Random plant samples from the three categories above were collected during this visit and then at approximate 14-day intervals throughout the season before being dispatched to CSL for PCR analysis. A final collection of tagged plants was made on 22 October along with a grab sample of approximately 200 untagged plants from the untreated area to determine the mean level of root distortion in the unsprayed area of crop.

The results for the molecular PCR testing indicated that there was a significantly higher (64 times) incidence of DNA of *P. farinosa* in the roots of the plants which had visible d. mildew on the foliage (category 2) than plants which appeared healthy at the time of tagging (category 1). This does tend to suggest perhaps that the d. mildew fungus is capable of moving into the hypocotyl/root tissues following crown infection. In the plants with leaf distortion and multiple crowning (category 3) there was a higher incidence of DNA of *P. farinosa* in the root tissues compared to the healthy plants (category 1) though the incidence was much lower than those in category 2. Approximately 7% of the 'healthy' tagged plants developed mild RMD symptoms and this is assumed to be due to the fact that there was either a latent infection at the time of tagging or else that they became infected later in the season. In comparison, 37% of those with visible d. mildew (category 2) and 45% of plants with leaf distortion, but no visible d.

mildew had mild RMD symptoms. This compared with a mean root distortion level of 14% across the remainder of the untreated area of the field. So, whilst this exercise has clearly demonstrated a close association between d. mildew infection, presence of DNA in the roots and RMD symptoms it was perhaps a little disappointing that the results were not much clearer. Ultimately, the aim must be to secure effective control of RMD in a season when the problem is severe using oomycete fungicides relative to an untreated control. Separately, it remains necessary to complete Koch's postulates and to reproduce RMD symptoms following specific inoculation of red beet with *P. farinosa* and then successfully re-isolate the fungus to prove cause and effect.

Financial Benefits

The financial benefits from this study cannot be fully determined until such time that the industry has the confidence that they can effectively control the problem with the application of oomycete or other fungicides. However, it is evident from 1998 and 2002 when RMD was exceptionally problematic for the UK industry that there is undoubtedly a financial prerogative to resolve this problem for red beet growers.

Action Points for Growers

- Continue to be aware of the risk from RMD in red beet and the potential economic significance should it occur.
- Monitor crops in early Spring for the first signs of downy mildew, root malformation or other possible symptoms that may be associated with the problem.
- Don't assume that because the problem hasn't been severe in the last 3 years that it won't recur at economically damaging levels in future crops.
- Until such time that we can be certain of the primary cause and can predict high risk periods for RMD i.e. what weather conditions are most conducive to RMD development it would be advisable, where possible, to develop a preventative disease control strategy.
- Only use beet seed from a reputable source and consider the potential risk of seed-borne disease.
- Use seed treatment containing metalaxyl-M e.g. Wakil XL, where possible, to minimise the risk from downy mildew and other potential pathogens from infecting the beet at emergence.
- Apply approved fungicides for downy mildew as part of a routine preventative programme, especially during periods of wet weather when conditions are conducive to the disease.
- Liaise closely with the Red Beet Technology Group and the HDC Technical Manager to ensure you have the latest information on fungicide availability for red beet.
- Where possible, provide continued support to ongoing research and development into RMD.

SCIENCE SECTION

Introduction

During early Autumn 1998 concerns were raised by a number of growers regarding the occurrence of an apparent new disorder or disease of red beet. As crops neared maturity roots were observed to be severely distorted. In addition to the distortion, affected roots had an elongated neck and, in some cases, had a thickened tap root. One particular characteristic of the affected beet was a russetting or corkiness around the shoulder of affected plants. The smaller or 'baby beet' size grades were reported to be particularly badly affected. The syndrome was referred to as root malformation disorder or RMD. Various estimates have put the economic losses due to RMD at around £1M/annum in years when the problem has been particularly severe (1998 & 2002).

Following the initial occurrence of RMD HDC sponsored an investigation at Stockbridge House during 1999-2001 to try and determine the cause for the symptoms. Studies initially commenced on a broad basis to conduct a literature search, distribute a questionnaire to growers, conduct a series of pot studies and to eliminate a number of possible factors that could potentially have led to such severe root distortion. During this initial investigation, tests for 'Rhizomania' and other virus diseases were conducted, as were tests for herbicide injury, nematode infestation and bacterial pathogens. All tests proved negative.

In the second year of the project information gleaned from the pot studies were used to design and undertake a series of replicated field-scale trials on commercial farms to evaluate the performance of various experimental fungicides applied as seed treatments and post-emergent HV sprays. Results from this work were more variable than hoped due largely to the relatively low incidence of RMD during that period. However, individual sites did respond moderately well to fungicides and at site 2 (Westwoodside) RMD symptoms were well controlled with metalaxyl-M applied as SL567 (for oomycete control) either as a seed treatment or drench application. At the other 2 sites levels of RMD were much lower. Some response from the applied products, particularly SL567A, Monceren (for *R. solani* control) and Biomex (also targeting *R. solani* primarily) was achieved. Whilst it was considered that further investigation was required to fully elucidate the problem, preliminary discussions with a view to extending the work for a 3rd year were not successful. Therefore, based on the 2-year study, it was concluded that the most probable cause for RMD was a *Pythium-Rhizoctonia* complex, infection occurring at the seedling stage with the distortion symptoms developing as the roots enlarged. A recommendation was therefore made to pursue On- or Off-Label authorisation for the fungicide metalaxyl-M (SL567) and possibly azoxystrobin (Amistar). Unfortunately though, for a variety of reasons, this recommendation was not taken forward by HDC and fungicide authorisations were not immediately secured.

In October 2002 growers, particularly in the Isle of Axholme region of South Yorkshire, again reported an extremely high incidence of RMD. On this occasion, it appeared that the problem developed quite late in the season (August-September). In some cases it was severe in fields that had not grown commercial crops in the Chenopodiaceae for several years or on land that had been down to grass for 20 years. As previously, the problem appeared to correlate closely with wet weather, in this case heavy rainfall during August after a prolonged dry spell. The reported absence of early symptoms and the presence of severe RMD in 'virgin' sites, rather than pointing to a soil-borne pathogen, tended to suggest aerial dissemination eg an aphid vectored virus or an air-borne fungus.

Close inspection of affected crops noted a fairly heavy infestation of downy mildew caused by *Peronospora farinosa* f. sp. *betae*, a pathogen not noted at particularly significant levels in previous years. As an oomycete fungus this obligate pathogen could also be expected to be well controlled (subject to the absence of resistant strains in the pathogen population) by SL567A. In other crops downy mildew fungi eg *Peronospora viciae* in peas are reported to infect seedlings systemically to cause distortion, without obvious sporulation. A web-based report from Oregon in the USA describes

symptoms of d. mildew in red beet that correlates closely with those of RMD and this certainly required further investigation.

A further project initiated in 2003 investigated the role played by both soil- and air-borne pathogens in the RMD problem in a series of field-scale trials as a further means of elucidating the primary cause. The primary objective was to evaluate a soil sterilisation treatment in conjunction with a range of existing and novel fungicides. Separately, a search of past scientific literature on the subject was conducted. The aim here was to determine if there was any information available to ascertain whether the d. mildew pathogen found on wild *Chenopodiaceae* possibly acted as a reservoir for subsequent infection of commercial 'beet', or indeed whether different host-specific pathovars were involved in the problem.

As a result of the mounting evidence of a possible association between infection by the d. mildew fungus (*P. farinosa*) and RMD symptoms discussions were opened with scientists at CSL. Following these initial discussions CSL and STC, during January-March 2004 tried to prove the hypothesis that the RMD affected roots were a result of a systemic invasion by the obligate oomycete pathogen *Peronospora farinose*, by developing a molecular (PCR) method for quantifying DNA of *P. farinosa* in red beet root tissues. Furthermore, an initial validation test using RMD affected and unaffected red beet roots gave very positive results and this further strengthened the case for an association between RMD and d. mildew infection.

As a result of this positive development and to maintain the impetus further work was commissioned by HDC in Spring 2004, designed to evaluate a range of fungicides aimed at d. mildew control for their efficacy and timing in controlling RMD in red beet and also to fully integrate the novel PCR technique for quantifying the d.mildew fungus in distorted roots. The aim was to identify two high risk commercial sites to establish fungicide comparison (x2) trials (superimposed over untreated and Wakil treated crops) and fungicide timing (x2) trials (in the non-Wakil treated area of the crops). Separately, occasional commercial crops, subject to the development of either d. mildew and/or RMD were monitored, to further investigate any causal relationship using the molecular technique developed at CSL. The results of the 2004 investigations are reported here.

Materials & Methods

(i) Trial site location

Following extensive discussion with industry representatives two sites for trial purposes were identified on commercial farms in South Yorkshire. At each site two separate trials looking at the efficacy of a range of fungicides and the timing of applications was established; resulting in 4 fully replicated trials in total.

At site 1 (Westwoodside) red beet seed cultivars Pablo (fungicide timings trial) and Crimson Globe (fungicide efficacy trial) were used whereas at site 2 (West Butterwick) cv. Pablo was used throughout. The trial crops selected were drilled during May as this is considered by many growers to represent the high risk period for RMD. In the fungicide comparison trials a range of fungicides and related treatments (Tables 1-2) were applied on a replicated basis approximately 2 weeks after drilling and at subsequent 2 week intervals throughout the season, the aim being to maintain fungicide protection against oomycete fungi throughout the growing season. In the adjacent fungicide timing trials a fungicide mixture (Fubol Gold + Invader) with proven activity against d. mildew was chosen to be applied at selected timings as per a pre-determined 2- or 4-spray schedule (Table 3). The fungicide mixture used in the timing trial was selected to counter any possible fungicide insensitive strains that may have been present in the pathogen population.

(ii) Fungicide Performance (Efficacy)

Fungicides for the comparative study were selected on the basis of known or reported activity against downy mildew or other oomycete fungi e.g. *Pythium* spp., *Phytophthora* spp. *or Albugo* spp. In most cases, the products chosen had UK approval for the control of blight (*Phytophthora infestans*) in potato. Two further alternating spray programmes were devised to represent a 'best case' experimental treatment programme (which included currently non-approved products) and a commercial programme using the best available, but currently approved, products. Treatments were applied on a 14-day spray interval to maintain good protection throughout the growing season.

Table 1 : Selected fungicides and programmes for the fungicide comparison trials in 2004

Product	Active Substance	Rate of application (product/ha)	Water volume (litres/ha)	No. & timing of applications
1. Untreated control	Water	-	-	max of 8, at 14 day intervals
2. Dithane	mancozeb	2kg	250	max of 8, at 14 day intervals
3. Fubol Gold	metalaxyl-M + mancozeb	1.9kg	250	max of 8, at 14 day intervals
4. SL567A	metalaxyl-M	0.2l	250	max of 8, at 14 day intervals
5. Invader	dimethomorph + mancozeb	2kg	250	max of 8, at 14 day intervals
6. Ranman Twinpack	cyazofamid	A – 0.2l B – 0.15l	250	max of 8, at 14 day intervals
7. Amistar	azoxystrobin	1.0	250	max of 8, at 14 day intervals
8. Shirlan	fluazinam	0.3l	250	max of 8, at 14 day intervals
9. Epok	fluazinam + metalaxyl-M	0.38l	250	max of 8, at 14 day intervals
10. DP98	Phosphonic acid	4l	250	max of 8, at 14 day intervals
11. Experimental programme	See table 2			
12. Commercial programme	See table 2			

Table 2 : Details of the experimental and commercial fungicide programmes (Treatments 11 and 12) used in 2004

Treatment	Treatment Programmes	Active Substance	Rate of application (product/ha)	Water volume (litres/ha)
11. Experimental programme	1. Fubol Gold	metalaxyl-M + mancozeb	1.9kg	250
	2. Amistar + Dithane*	azoxystrobin + mancozeb	1l + 2kg	
	3. Invader	dimethomorph + mancozeb	2kg	
	4. Fubol Gold	metalaxyl-M + mancozeb	1.9kg	
	5. Amistar + Dithane*	azoxystrobin + mancozeb	1l + 2kg	
	6. Ranman TP	Cyazofamid*	A – 0.2l, B – 0.15l	
	7. Invader	dimethomorph + mancozeb	2kg	
	8. Ranman TP	Cyazofamid*	A – 0.2l, B – 0.15l	
12. Commercial programme	1. SL567A	metalaxyl-M	0.6l	250
	2. Amistar	azoxystrobin	1l	
	3. Filex	propamocarb HCl	0.25l	
	4. SL567A	metalaxyl-M	0.25	
	5. Amistar	azoxystrobin	1l	
	6. SL567A	metalaxyl-M	0.25	
	7. Filex	propamocarb HCl	0.25l	
	8. SL567A	metalaxyl-M	0.2	

** The cyazofamid (Ranman) is provided as a 'Twinpack' and the rate of application is represented by A & B in the Table above.*

As part of a continuous monitoring programme, grab samples were collected from each untreated plot at both sites prior to each spray application. The collected plants were forwarded to CSL for Taqman PCR testing to monitor the level of DNA of the downy mildew fungus.

(iii) Fungicide Timing

In the fungicide timing trial a tank-mix containing Fubol Gold (metalaxyl-M + mancozeb) at 1.9kg/ha and Invader (Dimethomorph + mancozeb) at 2.0kg/ha was chosen to provide comprehensive activity against *P. farinosa*; from known activity against d. mildew in other crops. It was also chosen to counter any possible resistant strains in the pathogen population to either fungicide as they have contrasting modes of action and therefore cross-resistance is unlikely. Treatments were scheduled to be applied at approximate 14-day intervals to maintain good protection throughout the growing season.

Table 3 : Details of the proposed spray schedule in the fungicide timing trials in 2004

Application	June		July		August		September		October	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
1.	+	+	+	+	+	+	+	+	+*	+*
2.	+	+								
3.			+	+						
4.					+	+				
5.							+	+		
6.	+	+	+	+						
7.				+	+	+	+			
8.							+	+	+*	+*

** Fungicide treatments were terminated early at the end of September due to advance of crop maturity and the complete absence of d. mildew or RMD at the trial sites.*
All fungicide applications in the two trials comprised a tank-mix of Fubol Gold (1.9kg/ha) and Invader (2.0kg/ha) applied at 14 day intervals.

(iv) Crop Diary

Details of the various actions, treatments and assessments carried out in the various trial sites is outlined in Tables 4-5 below.

Table 4 : Crop diaries for the two* field trials designed to evaluate fungicide performance in 2004

Action	**Site 1 (Westwoodside)**	**Site 1a (Westwoodside)**	**Site 2 (West Butterwick)**
Drilling date	24 May	25 May	11 May
Cultivar	Pablo	Crimson Globe	Pablo
1st fungicide application	7 June	25 June	7 June
2nd fungicide application	-*	6 July	22 June
3rd fungicide application	-	20 July	6 July
Plant vigour & disease assessment	-	22 July	22 July
4th fungicide application	-	4 August	20 July
Disease assessment		6 August	
5th fungicide application		16 August	4 August
6th fungicide application		1 September	Trial terminated
7th fungicide application		24 September	
Harvest & final assessments	-	2 & 3 November	

*Trial site abandoned due to high levels of A*phanomyces.*Site 1 replaced with site 1a.

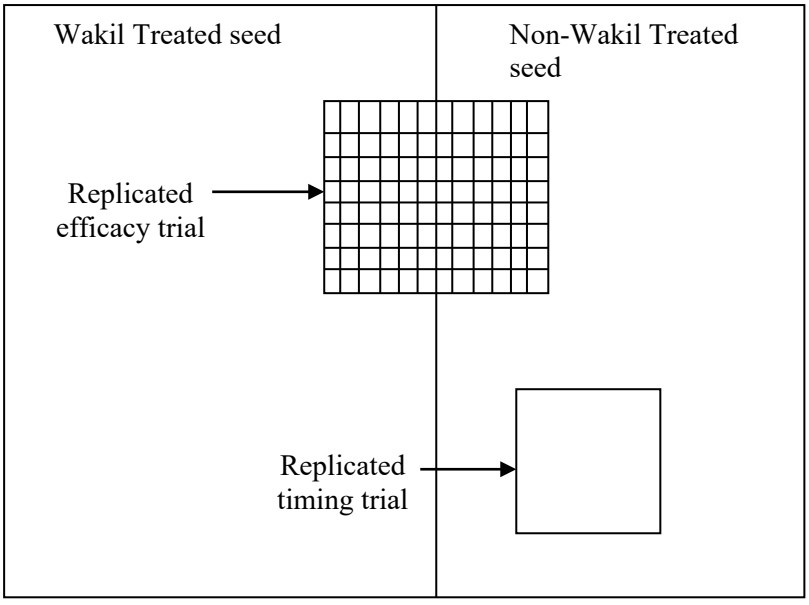
Table 5 : Crop diaries for the two field trials designed to evaluate fungicide timing in 2004

Action	Site 1 (Westwoodside)	Site 2 (West Butterwick)
Drilling date	24 May	11 May
Cultivar	Pablo	Pablo
1st fungicide application	7 June	7 June
2nd fungicide application	22 June	22 June
3rd fungicide application	6 July	6 July
4th fungicide application	20 July	20 July
5th fungicide application	4 August	4 August
Disease assessment	6 August	-
6th fungicide application	16 August	16 August
7th fungicide application	1 September	Trial terminated
8th fungicide application	24 September	
Harvest & final assessments	27 October	

(v) Trial Design

At each of the two commercial field sites (Westwoodside & West Butterwick) a fully randomised fungicide comparison trial, comprising 12 treatments with 4 replicates, was designed to be positioned with two replicates/treatment over Wakil treated seed, and two over untreated seed as shown in Figure 1 below. In the case of the fungicide timing trials, they were designed to be carried out in an adjacent area of the same crop but in the non-Wakil treated area.

Figure 1 : Layout of the proposed trials at the two commercial red beet crops in 2004



This design was fully employed at both sites initially. However, following a high incidence of *Aphanomyces cochlioides* in the fungicide comparison trial at site 1 (Westwoodside) the trial had to be abandoned though was immediately re-established in a nearby field. However, the replacement site only contained plants grown from Wakil treated seed and this had to be used instead. Fortunately, the area of the field where the original fungicide timing trial was located was less severely affected by *Aphanomyces* and did not need to be re-located.

(vi) Spray applications

In both the fungicide comparison and the timing trials sprays were coincided where possible and applied at approximate 14-day intervals in 250 litres water/hectare using an Oxford Precision sprayer with a 4-nozzle boom attachment and operating at 2-bar pressure. The first application was made within 2 weeks of sowing to provide protection to the young foliage as the efficacy of the Wakil seed dressing declined.

(vii) Assessment methods

All trial crops were monitored regularly (prior to each fungicide application) for the occurrence of any disease symptoms. Where symptoms of d. mildew, other significant pathogens or RMD were found detailed assessments were carried out. Random 'grab' samples were taken from the fungicide trial areas approximately 8 weeks post-sowing, to estimate plant vigour, the incidence of downy mildew, RMD or any other pathogens. At harvest a one-metre bed width was lifted, assessed for disease incidence and crop yields recorded. Throughout the trial period the experimental crops were monitored for the appearance of phytotoxicity or other symptoms and where present recorded and assessed. Details of the assessment scales used are presented below: -

a) Plant Vigour Indices

Plant vigour was assessed at Westwoodside during the final disease assessments, the following 0-3 scale was used:-

0 = Crop extremely poor, majority of foliage senesced prematurely
1 = Crop poor, thin and little green leaf tissue remaining
2 = Crop with average vigour, moderate level of leaf retention and leaf reddening
3 = Foliage very vigorous, with excellent leaf retention

A plot assessment of leaf disease was also carried out at Westwoodside during the final disease assessments due to the incidence of leaf-spot caused by *Cercospora beticola*. The following 0-3 scale was used:-

0 = No leaf spotting present, foliage healthy
1 = Low levels of leaf spotting present, majority of foliage healthy
2 = Moderate levels of leaf spotting present.
3 = Severe levels of leaf spotting present, majority of foliage affected.

b) RMD Index

See Appendix 3 for detailed assessment scale for RMD severity

Where appropriate, the various severity scales were converted to a 0-100 scale using the formula example below: -

$$\frac{0(0) + 1(1) + 2(2) + 3(3) + 4(4) + 5(5)}{\text{No. of plants or roots assessed}} \times \frac{100}{5}$$

c) Yield determination

Harvest assessments were carried out for both the fungicide performance and the fungicide timing trials. In each trial, a 1m strip spanning the entire bed was lifted for assessment. A detailed assessment of the number and severity of RMD affected roots was carried out and the weight of the roots (minus foliage) was recorded. This data was then used to calculate the approximate yield/ha.

(viii) Plant 'Tagging'

At one commercial site in late Spring 2004 a moderate-severe infection with d. mildew was reported by the grower. A visit to the site confirmed this diagnosis and it appeared to represent an ideal opportunity to monitor disease progression relative to RMD symptom development through the season. As the grower wished to protect the crop with fungicides appropriate advice was provided by BASIS qualified personnel on the understanding that a relatively small area of the crop in the centre of the field would remain unsprayed for experimental purposes. At a subsequent visit a large number of plants with 3 distinct symptoms were tagged using different marker canes so that symptom progression could be monitored over the season relative to RMD as determined by the PCR test. The plants were differentiated as follows:-

Category 1 : Healthy plants, no downy mildew or other symptoms visible
Category 2 : Early crown infection with downy mildew, sporulation clearly evident
Category 3 : Evidence of leaf distortion and multiple crown effect (some showing brown petiole symptom), but no sporulation of downy mildew

Plate 5 : Tagging at 'Tarmac' site

At approximate 2 weekly intervals sub-samples of the tagged plants were collected randomly, assessed for RMD or other symptoms and then dispatched to CSL for PCR testing. CSL held samples in cold storage until sufficient samples were available for a bulk analysis.

Other occasional *ad hoc* samples were also investigated using the molecular test during the 2004 season as they arose.

(ix) CSL Molecular Taqman PCR methodology

Assay design and optimisation

Probe and primers were designed to *Peronospora farinosa* 28S large subunit ribosomal RNA gene sequences obtained from public access databases (NCBI Accession no. AF235955) (Table 6). The assay was designed over a deletion site in the 28S gene that was present in *Peronospora* sp. but not in many fungi. However using current sequence information it was not possible to design a TaqMan assay specific to *P. farinosa* the sequences for other *Peronospora* species is identical and this assay will also detect them. In addition a previously designed internal control assay, which detects the Cytochrome oxidase gene (COX) in plant tissues was used as a normaliser for quantitation (Weller *et. al*. 2000).

Table 6: Primer and probe sequences of the *P. farinosa* and COX TaqMan assays

Oligo Name	Sequence 5′-3′
P. farinosa F	ATGGCTGCCGAGGAGGTA
P. farinosa R-B	GCGACGACTAGTCCACCAAG
P. farinosa Probe (Fam/MGB labelled)	AACGCAAGCGTAAGCC
COX F	CGTCGCATTCCAGATTATCCA
COX R_W	CAACTACGGATATATAAGRRCCRRAACTG
COX Probe (Joe/Tamra labelled)	AGGGCATTCCATCCAGCGTAAGCA

Initial optimisation of the *P. farinosa* assay was performed on DNA extracted from *P. violae* infected pansy leaves (supplied by Stockbridge Technology Centre). Experiments were carried out to establish the optimal primer concentrations in order to achieve greatest sensitivity. Small differences in the melting temperatures of primers, which would affect amplification efficiency, can be compensated for by altering the concentration of primers in the reaction. The two TaqMan assays, *P. farinosa* and COX, were multiplexed for use when testing red beet samples, both assays were performed in the same well reducing the cost of the test. However when testing fungal isolates from plates the *P. farinosa* assay is used in simplex.

Specificity

The specificity of the *P. farinosa* assay was tested against a range of fungal species and the Ct value recorded (Table 7). The Ct value is the cycle number at which the amount of fluorescence and therefore the amount of amplified DNA crosses a background level. The Ct value is inversely proportional to the amount of target DNA, lower Ct values indicate more target DNA present in the sample. When testing other species of fungi the Ct value can also be affected by mismatches in the primer or probe sequences.

For *P. farinosa* isolates, DNA was extracted from infected plant tissues, for all other isolates DNA was extracted from plate cultures. The *P. farinosa* assay was negative when used to test 4 *Fusarium* species, 4 *Phoma* species, *Rhizoctonia* solani and a species of *Pythium*. In addition 35 species of *Phytophthora* were tested including *P. erythroceptica*, *P. cryptogea* and *P. drechleri*. The 28S gene sequences for *Phytophthora* are very similar to *P. farinosa* sequences and therefore as expected, DNA from many *Phytophthora* species is amplified by the assay, however, the positive reactions seen when testing these species were, in most cases, much weaker, with higher Ct's than those obtained from *P. farinosa* isolates.

Table 7: TaqMan testing of Fungal species with the *P. farinosa* assay.

Species	Ct value
P.violae (from pansy leaves)	16.24
P. farinosa 2175 (dried leaves from 1975)	16.35
P. farinosa 90/19 (dried leaves from 1990)	16.91
Fusarium coeruleum	-
Fusarium sulphurium	-
Fusarium culmorum	-
Fusarium avenaceum	-
Phoma exigua var *foveata* (5 isolates tested)	-
Phoma lingam	-
Phoma herbarum	-
Phoma maevostonia	-
Rhizoctonia solani	-
Pythium sp.	-
Phytophthora nicotianae, P. drechleri (2 isolates*), P. botryose* (2 isolates*), P. clandestine, P. katsurae, P. lateralis, P. melonis, P. tenaculata, P. humicola, P. syringae* (2 isolates), *P. palmivora* (2 isolates)*, P. sojae, P. megasperma, P. cryptogea*	-
Phytophthora erythroceptica	30.21
Phytophthora erythroceptica	31.53
Phytophthora erythroceptica	31.18
Phytophthora citricola	29.02
Phytophthora pseudotsugae	25.86
Phytophthora citrophthora	25.33
Phytophthora arecae	30.86
Phytophthora heveae	28.69
Phytophthora sinenisi	30.38
Phytophthora inflata	27.79
Phytophthora porri	22.15
Phytophthora ilicis	28.34
Phytophthora frag. var. frag	30.56
Phytophthora cambivora	27.53
Phytophthora iranica	24.88
Phytophthora cactorum	24.24
Phytophthora gonapodyides	23.75
Phytophthora gonapodyides	22.74
Phytophthora cajani	29.20
Phytophthora capsici	27.15
Phytophthora colocasiae	30.01

DNA extraction

A comparison of two DNA extraction methods was carried out for the isolation of DNA from red beet. Firstly the CTAB method relies upon precipitation of DNA, which is then purified by several centrifuge steps, this method is commonly used for plant tissues and is robust, but is time consuming (taking up to 4 hours to extract a few samples) and labour intensive. In the second method DNA is bound to magnetic beads, which are then washed by transferring to a series of buffers, by the robotic Kingfisher system (Labsystems). This highly automated method is quicker, far less labour intensive and can be performed in 96 well plates for high-throughput testing. DNA was extracted from 20 red beets (supplied by Stockbridge Technology Centre) by each method. These DNA extracts were tested with the COX assay and the cycle threshold (Ct) values compared (Table 8).

Results from the two methods were highly comparable, the average Ct and therefore the average amount of DNA extracted in all samples are very similar. Either method could be used to effectively extract DNA from red beet, however, for the small number of samples tested here, the magnetic bead extraction method gave more consistent results indicating that this method is more reproducible. In

addition to this the highly automated nature of the magnetic bead method would prove more suitable for testing large numbers of samples. The magnetic bead method was selected as the DNA extraction method used when testing for *P. farinosa* in red beet.

Table 8: Comparison of DNA extraction methods

Sample Number	CTAB Method	Magnetic Bead Method
1	17.73	18.70
2	16.94	18.20
3	19.39	19.75
4	21.53	18.90
5	18.63	18.64
6	16.94	18.16
7	20.27	20.65
8	19.03	18.91
9	19.02	18.30
10	27.16	18.75
11	17.97	18.43
12	16.69	17.56
13	18.16	18.49
14	18.41	18.63
15	20.33	18.97
16	17.93	18.99
17	17.02	18.15
18	18.47	18.92
19	19.38	18.32
20	18.24	18.49
Mean of samples	18.96 (+/-2.24)	18.7(+/-0.62)

Sampling method
The distribution of *P. farinosa* within a plant was investigated to determine the best sampling method. Two red beets, showing symptoms of distortion, were sampled just beneath the skin; in the centre of the root; within the crown and within the base of the root. The DNA was extracted and all samples tested with the *P. farinosa* and COX assays. The amount of *P. farinosa* within the root proved to be highly variable, in the case of beet 2 testing just under the skin would have given a negative result however a sample taken from within the base of the same root would have given a positive result. In both cases the lowest Ct's, and therefore the highest levels of *P. farinosa* were found within the bottom of the root (Table 9).

(x) Quality Assurance

The study described was undertaken in accordance with the guidelines for Official Recognition of Efficacy Testing Organisations.

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(xi) Statistical Analysis

Data from the replicated trials was input into ARM 7 management software (Gylling Data Management) and analysed statistically. The Student-Newman-Keuls test was used (P=0.05) to provide a comparison of the results from the treated plots with the untreated. The results of these analyses are presented in the tables of results.

Results

(i) Fungicide comparison (Efficacy)

Trial Site 1 (Westwoodside)

The initial location chosen for this trial was unfortunately badly affected by root rotting and blackleg caused by the soil borne fungus *Aphanomyces cochlioides* during seedling emergence and establishment. The true severity of the infection really became apparent as the seedlings reached the 1st/2nd true leaf stage of development. As only one fungicide application had been carried out at this stage, a decision was made to abandon this site and transfer to a nearby field, which had been sown at a similar time. Unfortunately, the replacement site was all drilled with Wakil treated seed and a comparison with untreated seed was no longer possible at this site as originally planned.

The trial area was monitored for d. mildew infection prior to each fungicide application (every 14 days). Only trace levels of d. mildew were observed on the plants in the trial area, or on those in surrounding fields (G. Smith *pers. comm*.). A 'grab' sample was collected from each of the untreated plots (at the ends of the plots) for molecular monitoring and analysis to ascertain the onset of any downy mildew infection prior to each spray application.

Table 9: Mean quantity of *P. farinosa* DNA detected in roots from untreated plots during the trial period.

Date of sample	Westwoodside	West Butterwick
22 June 2004	-	0.0005
25 June	0.0006	-
6 July	1.0133	0.0004
20 July	0.0003	0.0001
4 August	0.9636	0.1395
16 August	2.6584	-
1 September	1.2038	-
24 September	0.0207	-

Quantity of *P. farinosa* per beet relative to positive control of value 1

These results illustrate that negligible levels of d. mildew DNA were detected in the roots collected at the West Butterwick site. On three occasions, at Westwoodside, the mean quantity of *P. farinosa* DNA in roots was equal to, or slightly higher than the positive control used in the PCR assay. This suggests that despite the almost complete lack of visual sporulation of d. mildew on the foliage of the plants, d. mildew was present in the roots, albeit at relatively low levels.

The full number of scheduled fungicide applications were applied to the trial area. Further random 'grab' sampling comprising approximately 10-12 plants/plot was carried out on the 22 July to determine the relative incidence and severity of different symptoms in the crop. Assessments of plant height, root diameter, incidence and severity of RMD, incidence of the brown petiole symptom, and incidence of visible downy mildew were all made at this time (Table 10).

Table 10: Agronomic and disease assessments in the fungicide comparison trial at Westwoodside on 22 July 2004

Treatment	Plant Height (cm)	Root Diameter (cm)	RMD Mean Severity (0-100 index)	Mean Incidence of Brown Petiole/plot	Mean Incidence of sporulating DM/plot
1. Untreated control	47.5[a]	2.5[a]	0	0.5[a]	0[a]
2. Dithane	44.7[a]	2.2[a]	0	0.6[a]	0[a]
3. Fubol Gold	48.3[a]	2.7[a]	0	0.5[a]	0[a]
4. SL567A	45.9[a]	2.2[a]	0	0.5[a]	0[a]
5. Invader	48.2[a]	2.4[a]	0	0.5[a]	0.02[a]
6. Ranman Twinpack	44.2[a]	2.0[a]	0	0.3[a]	0.02[a]
7. Amistar	48.3[a]	2.4[a]	0	0.4[a]	0[a]
8. Shirlan	48.1[a]	2.3[a]	0	0.5[a]	0[a]
9. Epok	47.3[a]	2.2[a]	0	0.5[a]	0[a]
10. DP98	46.3[a]	2.3[a]	0	0.4[a]	0[a]
11. Comm. Programme	49.1[a]	2.6[a]	0	0.6[a]	0[a]
12. Exp. Programme	48.0[a]	2.4[a]	0	0.4[a]	0.02[a]
LSD (P=0.05)	3.5	0.4	0	0.2	0.03
Standard Deviation	2.4	0.3	0	0.2	0.02
Co-efficient of Variance	5.1	11.2	0	33.5	395.94

Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls)

No differences were found between any of the treatments in terms of vigour or crop development at this interim assessment. Similar levels of incidence of the brown petiole symptom were seen across the treatments. Only a few of the plants assessed had sporulating downy mildew present at this stage in the trial crop.

Routine crop monitoring continued to be made and a final detailed assessment of crop vigour, disease and yield was made on the 3 November 2004 (Table 11). By this stage, a notable, and significant difference in crop vigour was observed though this did not correlate well with the presence of d. mildew or RMD symptoms in the trial site. Instead, the results appeared to relate to the incidental control of leaf-spot caused by *Cercospora beticola* that had developed in this crop during the growing season (Plate 6).

Table 11 : Final assessment of crop vigour , disease and yield at harvest of the fungicide comparison trial at Westwoodside on 3 November 2004

Treatment	Plot Vigour (0-3 scale)	Mean severity of *Cercospora* leaf spot (0-3 scale)	Total No. of distorted roots/treatment	Mean severity of RMD distortion (0-5 scale)*	Mean Yield (tonnes/ha)
1. Untreated control	1.0^{c}	2.8^{a}	27^{a}	0.19^{a}	81.4^{a}
2. Dithane	2.5^{ab}	1.5^{cd}	12^{d}	0.10^{a}	87.4^{a}
3. Fubol Gold	2.3^{b}	1.8^{bcd}	12^{d}	0.07^{a}	85.4^{a}
4. SL567A	1.0^{c}	3.0^{a}	2^{i}	0.03^{a}	85.8^{a}
5. Invader	2.3^{b}	1.5^{cd}	14^{c}	0.10^{a}	84.2^{a}
6. Ranman Twinpack	1.0^{c}	3.0^{a}	10^{e}	0.09^{a}	80.9^{a}
7. Amistar	3.0^{a}	1.0^{d}	10^{e}	0.08^{a}	82.7^{a}
8. Shirlan	2.3^{b}	2.5^{ab}	15^{b}	0.12^{a}	87.7^{a}
9. Epok	2.0^{b}	2.8^{a}	9^{f}	0.07^{a}	91.0^{a}
10. DP98	1.3^{c}	2.8^{a}	12^{d}	0.06^{a}	88.8^{a}
11. Exp. Programme	2.5^{ab}	1.5^{cd}	6^{g}	0.06^{a}	86.4^{a}
12. Comm. Programme	2.3^{b}	2.3^{abc}	3^{h}	0.02^{a}	86.3^{a}
LSD (P=0.05)	0.45	0.64	-	0.12	-
Standard Deviation	0.31	0.45	-	0.08	-
Co-efficient of Variance	16.0	20.3	-	104.3	-

Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls)
* Calculated on a mean sample size of 50 roots/plot.

The lowest level of *Cercospora* leaf spot and the corresponding best plant vigour were seen following treatment with Amistar (T7) with treatments 2, 3, 5 and 11 also showing low levels of leaf spot and good vigour. This is likely, in most cases, to be attributed to the protectant qualities of the broad spectrum fungicide mancozeb present in many of the formulated 'blight' products. Unfortunately, other foliar pathogens, including downy mildew, were not present in the crop at this assessment.

A plot-wide 1m strip was therefore harvested from each plot to allow detailed examination of roots for RMD and other symptoms and to conduct a yield determination. In this assessment low levels of RMD affected roots were found in the majority of plots, but the severity of the symptoms was very mild. There were however significant differences between the treatments with regard to the total number of affected roots (Table 9) and, in comparison to the untreated control plots, all the fungicide treatments (designed to control oomycete fungi) provided a statistically significant reduction in root distortion. The highest number of RMD affected roots (27) was recorded in the untreated plots, whilst SL567A and the Commercial programme both resulted in a highly significant reduction in the number of distorted roots (2 and 3 roots respectively). All of the other blight fungicides also had a significantly lower incidence of distorted roots compared to the untreated control. However, there was no significant difference in the mean severity of the distortion (even though the overall trend was similar) and this is probably a reflection of the mild distortion symptoms found on the affected roots. No significant differences in yield were observed between the treatments and this provides strong evidence to suggest that none of the applied intensive fungicide programmes were phytotoxic and certainly, throughout this trial duration, no symptoms of phytotoxicity were recorded following application of any of the fungicide treatments.

Plate 6: Prolonged greening and improved plot vigour in treated plots, November 2004

Trial Site 2 (West Butterwick)

The trial crop at West Butterwick established exceptionally well and plants from this site showed increased growth and vigour compared to those at site 1/1a. An interim assessment from random 'grab' samples in the plots was carried out on 22 July (Table 12).

Table 12: Interim agronomic and disease assessment on 22 July 2004

Treatment	Plant Height (cm)	Root Diameter (cm)	RMD Mean Severity (0-100 index)	Mean Incidence of Brown Petiole/plot	Mean Incidence of sporulating DM/plot
1. Untreated control	65.6[a]	4.5[a]	0	0.8[a]	0
2. Dithane	64.4[a]	4.3[a]	0	0.7[a]	0
3. Fubol Gold	65.7[a]	4.4[a]	0	0.6[a]	0
4. SL567A	65.7[a]	4.6[a]	0	0.7[a]	0
5. Invader	66.8[a]	4.9[a]	0	0.9[a]	0
6.Ranman Twinpack	64.7[a]	4.7[a]	0	0.7[a]	0
7.Amistar	66.5[a]	4.7[a]	0	0.8[a]	0
8. Shirlan	66.9[a]	4.9[a]	0	0.7[a]	0
9. Epok	66.1[a]	4.5[a]	0	0.7[a]	0
10. DP98	65.7[a]	4.8[a]	0	0.8[a]	0
11. Comm. Programme	66.0[a]	4.8[a]	0	0.7[a]	0
12. Exp. Programme	67.6[a]	4.7[a]	0	0.7[a]	0
LSD (P=0.05)	4.1	0.7	0	0.3	0
Standard Deviation	2.8	0.5	0	0.2	0
Co-efficient of Variance	4.3	10.4	0	25.3	0

Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls)

Plants were on average 20cm taller and the roots were twice the diameter of those at site 1, although the slightly earlier sowing date would have some influence on this data. Similar levels of the brown petiole symptom were recorded at this site compared to Site 1. No RMD affected roots or any visible downy mildew infection was recorded at this time.

Regular monitoring of the crop, prior to each fungicide application was carried out throughout the season and samples were collected from the untreated plots for molecular testing (Table 9). A

thorough examination of the crop was carried out at the end of August 2004 as the remainder of the field was already mature and about to be harvested. There was no evidence of downy mildew infection, root distortion, plot vigour differences or phytotoxicity symptoms at this time. As a result of the detailed observations conducted a decision was taken to terminate further fungicide applications at this site. However, the trial site continued to be monitored into the autumn in case of any late development with respect to RMD. A further but final visit to the site in late autumn indicated that there was still no d. mildew or incidence of root distortion (RMD) and a decision was therefore made to terminate the trial site and no further assessments or harvesting were carried out.

(ii) Fungicide timing

Trial Site 1 (Westwoodside)

The fungicide timing trial at Westwoodside was located in the non-Wakil XL treated area of the same field as the initial fungicide comparison trial. However, as reported above, due to an unexpected but patchy infection with *Aphanomyces* in this field the fungicide comparison trial had to be re-located into an adjacent field. Fortunately, the fungicide timing trial was in an area of the field largely unaffected by *Aphanomyces* and as such the trial was retained *in situ* throughout the season. As before, careful inspection of the site prior to the fortnightly fungicide applications was carried out. However, no natural infection with downy mildew was observed during routine monitoring through the season. Neither was there any obvious indication of RMD development in any of the trial plots. All the plots were harvested and assessed on the 27 October. Similar yields were seen throughout and only a trace number of roots (only 6 over the trial area) were found which exhibited mild distortion symptoms. There was no specific pattern to the few distorted roots that were found.

Trial Site 2 (West Butterwick)

As with the adjacent fungicide comparison trial at this site, the plants flourished and matured rapidly. Throughout the season downy mildew was not detected at all and no symptoms of RMD were observed throughout the trial period. At maturity there were no visible differences between plots and neither were there any adverse symptoms, including phytotoxicity. The trial was therefore terminated and no further assessments were taken at maturity.

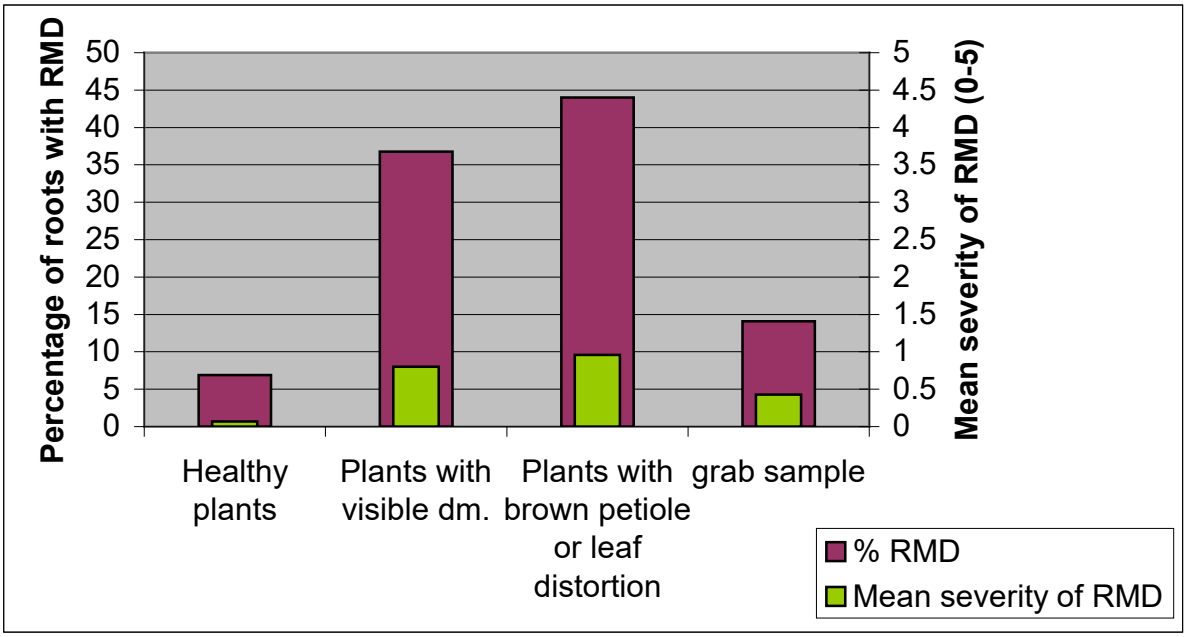
iii) Plant 'Tagging' in 2004

Following on from observations made in 2003, from discussions with industry representatives and in light of the new PCR technique for quantifying DNA of *P. farinosa* in the root tissues of red beet, we asked grower-members of the Red Beet Technology Group to contact us if they noticed downy mildew, or indeed RMD in any of their crops. A site ('Tarmac' field) being rented by one RBTG member was brought to our attention in early June, with a moderate downy mildew infection. In an unsprayed area in the centre of the crop extensive tagging of three specific foliar features was carried out i.e. 1. healthy plants, 2. obvious crown infection with d. mildew and 3. leaf distortion, multiple crowning but no d. mildew. Sub-samples of each batch of 'tagged' plants were collected on a fortnightly basis and dispatched to CSL for molecular testing.

During a final visit to the 'Tarmac' field site on the 25 October, the remaining tagged plants were collected along with a random 'grab' sample of approximately 200 plants collected from within the unsprayed (but not tagged) area of the field. A detailed assessment of the level of RMD and other symptoms on all of the roots was carried out prior to forwarding to CSL for the Taqman PCR analysis. Figure 2 shows the incidence of RMD and the mean severity of the symptom in each of the samples gathered.

The data suggests that the observation of visible d. mildew, or of other unusual crown or foliage symptoms does correlate well with the development of RMD affected roots. The plants identified as 'healthy' during the initial tagging procedure resulted in the lowest incidence and severity of RMD overall, this corresponds with the grab sample of untagged plants which showed a higher incidence of RMD than the 'healthy' plants, but lower than the lants tagged with visible or unusual crown symptoms. The grab sample is, in effect, a mixture of all of the possible variations, from healthy to those plants showing severe d. mildew infection.

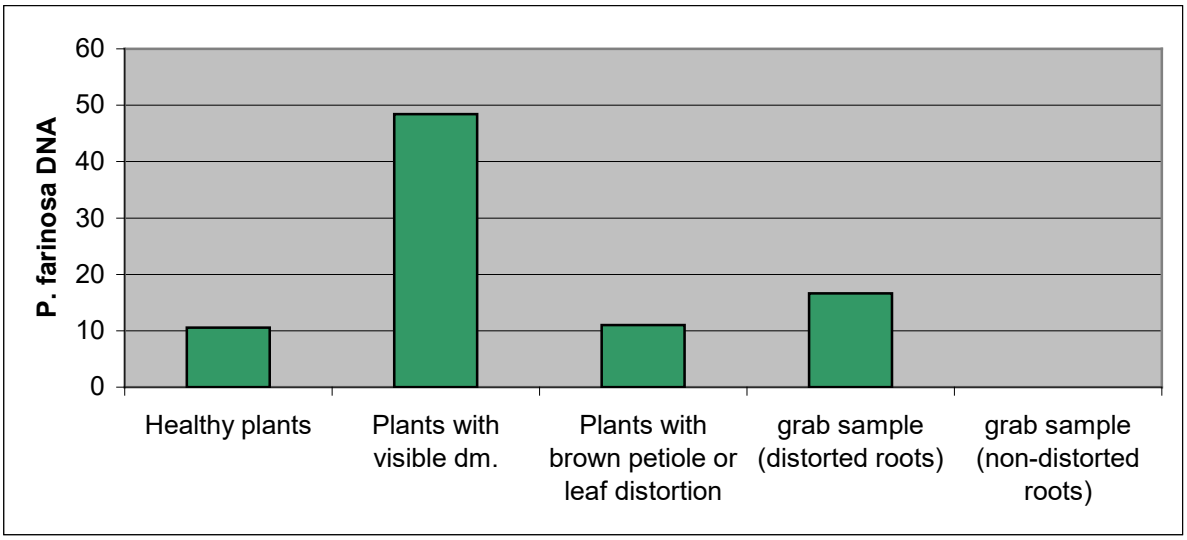
Figure 2. Incidence and severity of RMD affected roots in the tagged plant samples collected from the 'Tarmac' field site on 25 October 2004



There was a moderate-good correlation between the number of healthy roots that had been tagged and the low incidence of RMD (7%). The low incidence of RMD in the healthy plants could perhaps be accounted for by the presence of latent (symptomless) infection with d. mildew at the time of tagging or alternatively a later infection by *P. farinosa* after the plants had been tagged. In contrast, where plants were tagged with obvious crown infection at an early stage in crop development a much higher incidence of roots had RMD symptoms, albeit with relatively mild symptoms. Of particular interest was that the remaining tagged plants with other more unusual symptoms of leaf distortion, multiple crowning, brown petioles (but no sporulation of d. mildew) had an even higher incidence of root distortion, consistent with a mild form of RMD. Relative to the large random 'grab' sample taken at this time we were evidently selecting out tagged plants with an increased propensity for root distortion (Figure 2).

If the hypothesis is correct that d. mildew infection of the root tissues is responsible for the RMD symptoms observed sporadically in red beet crops then the molecular analysis of the root tissues at CSL should confirm this. However, whilst there was a very good correlation with the recovery of *P. farinosa* DNA from the healthy and *P. farinosa* infected tagged plants (Categories 1 & 2), the correlation with the more unusual distortion symptoms (Category 3) was less evident and the DNA recovery level was the same as that for the healthy beet (Figure 3). On a slightly more positive note, in the larger 'grab' sample from this site, two sub-samples of 10 roots with or without root distortion were analysed using the PCR technique and found to have a marked contrast in terms of DNA presence. The distorted roots had a positive DNA recovery whereas DNA was not recovered from the non-distorted roots in the 'grab' sample (Figure 3).

Figure 3. Determination and quantification of DNA of *P. farinosa* in 'tagged' plants from the 'Tarmac' field site on 25 October 2004.



*Quantity of *P. farinosa* DNA is shown as multiples of that found in a reference positive control, given a value of 1.

Further assessments carried out on the 'grab' sampled plants from the 'Tarmac' field also revealed further interesting data. Assessments were carried out on approximately 200 roots, of these roots 14% were affected by RMD. This percentage is much higher than had been recorded on any commercial crops in the area, and may reflect the lack of fungicides applied to this trial area. Only one plant had visibly sporulating d. mildew present on the foliage, however this root was severely distorted. Approximately 13.5% of the plants exhibited leaf distortion; a possible symptom of a systemic d. mildew infection, of these 58% had RMD. Almost 6% of the plants had the brown petiole symptom, which had been linked with distorted roots previously, of these, almost 64% had RMD. This information suggests correlations between foliar symptoms and RMD.

(iv) Molecular Testing at CSL in 2004

Quantitation of *P. farinosa* on known distorted and healthy Red Beet roots

The use of a normaliser assay allowed for any differences in sampling, extraction efficiency or amount of DNA added to the plate. Each sample was tested with both the *P. farinosa* assay and the COX assay and the results presented relative to the amount of plant tissue present. DNA was extracted from within the roots of 10 healthy and 10 affected (distorted) red beet and the amount of *P. farinosa* present was quantified using the Comparative Ct method and presented relative to a positive pansy sample (Table 14).

High amounts of *P. farinosa* were found in red beets showing symptoms of distortion, whereas very little or no *P. farinosa* was found in the non-symptomatic samples.

Table 13: Distribution of *P. farinosa* within red beet roots

		Distorted beet 1	Distorted beet 2
P. farinosa assay	Under skin of beet	32.21	40.00
	Centre of beet	28.86	33.11
	Within crown	28.19	27.94
	Within the base of the root	24.74	27.20
Cox assay	Under skin of beet	16.87	18.55
	Centre of beet	19.28	28.51
	Within crown	20.28	18.05
	Within the base of the root	16.89	16.79

Table 14: Quantity of *P. farinosa* in 10 healthy and 10 affected red beet samples relative to an arbitrary positive control.

Sample Name	*Quantity of P. farinosa DNA relative to positive pansy sample*
Healthy 1	0
Healthy 2	0.22
Healthy 3	0
Healthy 4	0
Healthy 5	0.24
Healthy 6	0
Healthy 7	0
Healthy 8	0
Healthy 9	0
Healthy 10	0
Affected 1	13048.25
Affected 2	14782.15
Affected 3	1712.12
Affected 4	16804.60
Affected 5	1448942.15
Affected 6	3043.61
Affected 7	17948.41
Affected 8	3424.24
Affected 9	1324.81
Affected 10	3799.43

Verification of *P. farinosa* in distorted red beet

Gel based PCR was carried out on DNA from healthy and affected beets using primers designed by Petersen and Rosendahl (2000). These primers were designed to amplify a 1.12kbp portion of DNA from the large subunit ribosomal RNA region. A band of approximately 1.12kbp was amplified from 7 of the affected beets and also *P. farinosa* pansy leaves and isolate 2175. This band was not observed when PCR was carried out on healthy beets. The DNA band amplified from affected beet (Nos. 1, 4 and 5) was sequenced and compared to sequences of *P. farinosa* and other fungi from public access databases, using a Clustal V alignment in the Megalign program (DNAstar). These sequences had a high degree of sequence homology (data not shown) with a known *P. farinosa* sequence supporting the diagnosis of *P. farinosa* in distorted red beet.

Quantitation of *P. farinosa* in field samples

DNA was extracted from 550 field samples of red beet using the magnetic bead method. These samples included the untreated control samples collected prior to each spray application, roots from the 'tarmac' site, and also samples collected during the final harvest assessment of the fungicide efficacy trial at Westwoodside. These extracts were tested with the *P. farinosa* and COX TaqMan assays in multiplex. Previous *P. farinosa* positive red beet DNA extracts (Affected 1-10) were pooled

and used as a positive control and added to each plate. The Comparative Ct method (Applied Biosystems) was used to quantify the amount of *P. farinosa* relative to the positive control. Full-tabulated data for all samples tested by Taqman PCR are shown in Appendix 4.

Discussion

The cause of root malformation disorder has proved to be a difficult problem to solve. In the normal course of field trials the researcher has a known pathogen and an obvious visible disorder to monitor. A range of pesticide spray applications, or other treatments, can then be applied and the effect of these treatments recorded and analysed. Working with any fungal, bacterial or insect pest carries with it a range of difficulties in terms of relying on natural infection versus artificial inoculation or infestation, climatic influence on the pest or disease, and the natural cycle of pest and disease pressures. The work on RMD has proved more difficult as when the problem of root distortion in red beet was initially brought to our attention in 1998 information regarding possible causal agents was very scarce and since then the problem has proved to be highly sporadic and seasonal in its appearance. Initial work by STC investigated possible links with soil-borne fungi such as *Pythium* spp. and *Rhizoctonia solani;* though to date no clear evidence to support their direct role in RMD has been established.

Observations made by one grower of a high incidence of downy mildew in red beet crops in years when RMD was particularly severe led STC scientists to consider the possibility that an infection with this pathogen may be the primary cause of the root distortion in red beet. As this organism is an obligate pathogen (it cannot be cultured) it has been necessary to rely solely on the incidence of natural infection in commercial crops. Downy mildew is an oomycete fungus which has quite specific climatic requirements for its survival, it requires cool, damp conditions e.g. normal spring or autumn weather for spread and survival. Initial studies in 2003 focused on using a range of fungicide programmes to control specific pathogens and hopefully elucidate the causal agent by eliminating it from some treatments and reducing the incidence of RMD. Very limited information was gained from these trials, as the incidence of RMD proved relatively low compared to the previous year.

In a further effort to establish a link between RMD and downy mildew HDC funded Central Science Laboratory (CSL) to develop a molecular bioassay (Taqman PCR) to quantify any downy mildew DNA that might be present in distorted roots. The results (Table 10) were exceptionally clear and very encouraging. This provided a clear focus as to the possible cause of RMD and helped 'steer' subsequent work.

The work carried out by STC in 2004 on grower sites was aimed at investigating the efficacy of a broad range of fungicides specifically against d. mildew (*P. farinosa)*. A further trials series also looked at the most effective timing of fungicide applications to see if a small no. of well timed sprays would be sufficient to prevent root distortion. However, despite careful monitoring and sampling at each of the sites we have progressed little due to the almost complete absence of downy mildew and RMD in red beet crops during 2004. This is considered to be most likely due to the warm, dry spring weather which was not suitable for initial infection and subsequent spread of the downy mildew pathogen. The fungicide efficacy trial at Westwoodside did provide some indication that application of oomycete fungicides can have a significant effect in terms of reducing the number of roots with RMD compared to an untreated control. Products such as SL567A and the commercial programme resulted in very low numbers of RMD affected roots.

We were able to employ the new molecular test developed by CSL to monitor the trial area crops and also to undertake a large and detailed plant 'tagging' investigation on a separate commercial crop in the Isle of Axholme area. Results obtained from this testing and other *ad hoc* samples during the season continue to show good correlation of a link between RMD and d. mildew. The regular monitoring and continued communication with industry representatives which suggested a limited d. mildew infection and a low incidence of RMD (< 1-2% roots affected) also adds further support to the hypothesis that a systemic infection by *P. farinosa* is responsible for the observed root distortion in red beet.

Commercial red beet growers are now employing several strategies to reduce infection with downy mildew e.g. using Wakil treated seed, monitoring crops closely, applying metalaxyl and strobilurin based fungicides to prevent, and hopefully control infection. All of these strategies will serve to

reduce the inoculum load of d. mildew (i.e. reduce spore production) and further reduce the chance of infection in the crop. This may have had a very beneficial affect during 2004 when disease levels were relatively low though they may not be sufficient in other seasons when climatic factors are more suited to the pathogen.

It is therefore recommended that additional work on RMD be continued in 2005 to further investigate methods to reduce the incidence and severity of root distortion or RMD by controlling downy mildew. Priorities for study continue to be demonstrating Kochs postulates via laboratory based artificial inoculation studies, to demonstrate effective control of d. mildew and RMD in the field through a series of well-timed fungicide applications and to ensure continued availability of suitable protectant and eradicant fungicides to develop an effective fungicide programme for the crop, whilst minimising he risk of fungicide resistance developing. The possibility of some or all of this work being undertaken as part of a PhD studentship should be considered.

Conclusions

- Downy mildew was generally present at low to negligible levels in commercial crops during Spring-Summer 2004. Occasional crops were found with moderate infection levels in late Spring though a change to drier weather prevented further development of the disease.

- RMD levels were very low commercially in 2004 and reports from growers suggested that typically less than 1-2% of graded roots were affected with root distortion and indirectly this supports the hypothesis of a possible link between early systemic infection by *P. farinosa* and RMD development on the roots later in the season.

- Two fungicide efficacy trials and 2 fungicide timing trials were established successfully at 2 grower sites in 2004. Unfortunately, an early infection with *Aphanomyces cochlioides* at one site required a relocation of that trial.

- Both the fungicide efficacy and timings trial crops conducted at Site 2 in West Butterwick remained strong & healthy crop and no d. mildew or RMD developed during the season. The surrounding commercial crop matured early and following a thorough examination of the trial areas it was decided to discontinue the spray programme slightly earlier than originally scheduled and ultimately both trials were abandoned.

- Useful information was gathered from the fungicide efficacy trial at Site 1 (Westwoodside). . Whilst little d. mildew was observed in the crop during the season significant differences in terms of the number of distorted beet in each treatment were observed during harvest; although the overall severity of the distortion was low. All of the oomycete fungicides applied resulted in a reduction in the number of distorted beet compared to the untreated control, with SL567A and the commercial programme resulting in the lowest incidence of RMD affected roots.

- At the same site there was a difference in terms of plant vigour and continued leaf greening. The effect was seen most clearly in plots treated with Amistar and several of the other fungicide treatments, all of which contained mancozeb. The increased plant vigour corresponded with significant reductions in the incidence of leaf-spot caused by *Cercospora beticola*.

- Crop monitoring, plant tagging and molecular analysis of root tissues from plants at the commercial 'Tarmac' site provided additional useful information and further, albeit limited, evidence of the correlation between RMD and downy mildew was gained.

- No evidence of any phytotoxicity effects was seen on plants at any of the trial sites following application of the various experimental fungicide treatments and/or programmes.

Technology Transfer

As in previous years the information from this project has been relayed to the industry throughout the season via one-to-one contact with growers, via meetings of the red beet technology group and the various activities of the Chairman Mr Graham Smith and the project team.

References

Byford, W J (1967). Host specialisation of *Peronospora farinosa* on *Beta*, *Spinacia* and *Chenopodium*. *Transactions of the British Mycological Society* **50** (4), 603-607.

Cook, H T (1936).Cross-inoculation and morphological studies on the *Peronospora* species occurring on *Chenopodium album* and *Spinacia oleracea*. *Phytopathology* **26**, 89-90.

Danielson, S (2001). Heterothallism in *Peronospora farinosa* f. sp. *chenopodii*, the causal agent of downy mildew of quinoa (*Chenopodium quinoa*). *Journal of Basic Microbiology* **41** (5), 305-308.

Danielson, S; Bonifacio, A & T Ames (2003). Diseases of quinoa (*Chenopodium quinoa*). *Food Reviews International* **19** (1-2), 43-59.

Dzhanuzakov, A (1962). Specialisation and variability in some Peronosporaceous fungi. *Bot.Zh. SSSR* **47**, 862-866. (In *Review of Applied Mycology* **42**, 4 (1963)).

Frinking, H D; Harrewijn, J L & C F Geerds (1985). Factors governing oospore production by *Peronospora farinosa* f sp *spinaciae* in cotyledons of spinach. *Netherlands Journal of Plant Pathology* **91** (5), 215-223.

Frinking, H D & E G A Landers (1986).A comparison of two pathosystems; downy mildew on *Spinacia oleracea* and on *Chenopodium album*. *Netherlands Journal of Plant Pathology* **92** (3), 97-106.

Fuckel, L (1865). *Peronospora schactii* n.sp. *Fung. Rhen*. 1508.

Hiura, M (1929). Studies on some downy mildews of agricultural plants III. On the downy mildew of spinach. *Agriculture Hort.., Tokyo* **4**, 1394-1406.

Irish, B M; Correll, J C; Koike, S T; Schafer, J & T E Morelock (2003). Identification and cultivar reaction to three new races of the spinach downy mildew pathogen from the United States and Europe. *Plant Disease* **87** (5), 567-572.

Leach, L D (1931). Downy mildew of the beet caused by *Peronospora schactii* Fuckel. *Hilgardia* **6**, 203-251.

Petersen AB, Rosendahl S (2000). Phylogeny of the Peronsporomycetes (Oomycota) based on partial sequences of the large ribosomal subunit (LSU rDNA). *Mycological Researc*h, **104** (11), 1295-1303.

Spencer, D M (1981). The downy mildews. Academic Press, London. 636 pp.

Vanasch M A J & H D Frinking (1988). Heterothallism in *Peronospora farinosa* f. sp *spinaciae*. *Transactions of the British Mycological Society* **91**, 692-693.

Weller SA, Elphinstone JG, Smith NC, Boonham N, Stead DE (2000). Detection of Ralstonia solanacearum strains with a quantitative, multiplex, real-time, fluorogenic PCR (TaqMan) assay. *Applied and Environmental Microbiology*, **66** (7), 2853-2858 JUL.

Wright, C M & W D Yerkes (1950). Observations on the overwintering of the pathogen causing downy mildew of spinach in the Wala-Walla area. *Plant Disease Reporter* **34**, 28.

Yerkes, W D & C G Shaw (1959). Taxonomy of the *Peronospora* species on *Cruciferae* and *Chenopodiaceae*. *Phytopathology* **49**, 499-507.

Acknowledgements

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Appendices

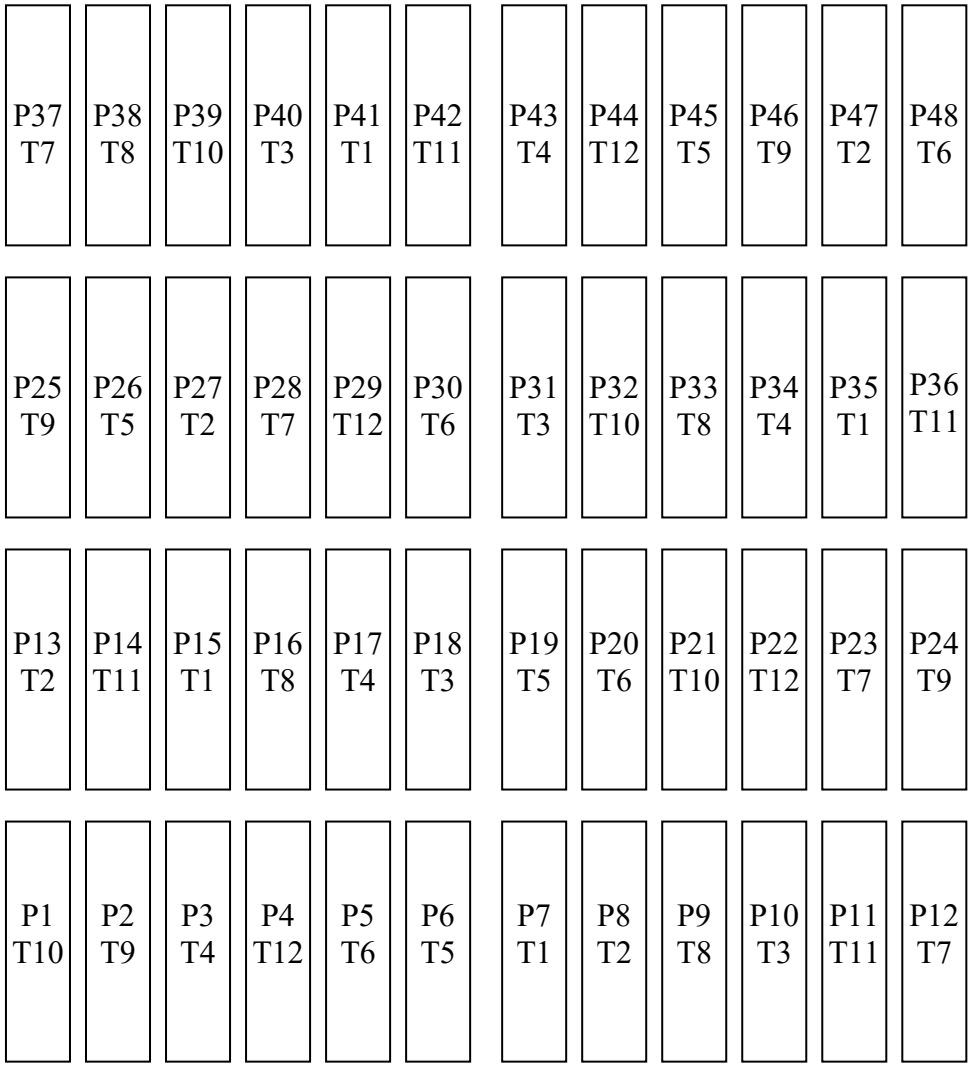
Appendix 1a : Trial plan – Site 1 Fungicide Trial
Appendix 1b : Trial plan – Site 2 Fungicide Trial

Appendix 2a : Trial plan – Site 1 Timings Trial
Appendix 2b : Trial plan – Site 2 Timings Trial

Appendix 3 : Assessment scale for RMD symptoms

Appendix 4 : Tabulated data from Taqman PCR testing

RMD in Red Beet – 2004 Trials E145a
Fungicide Trial Plan Carr Farm

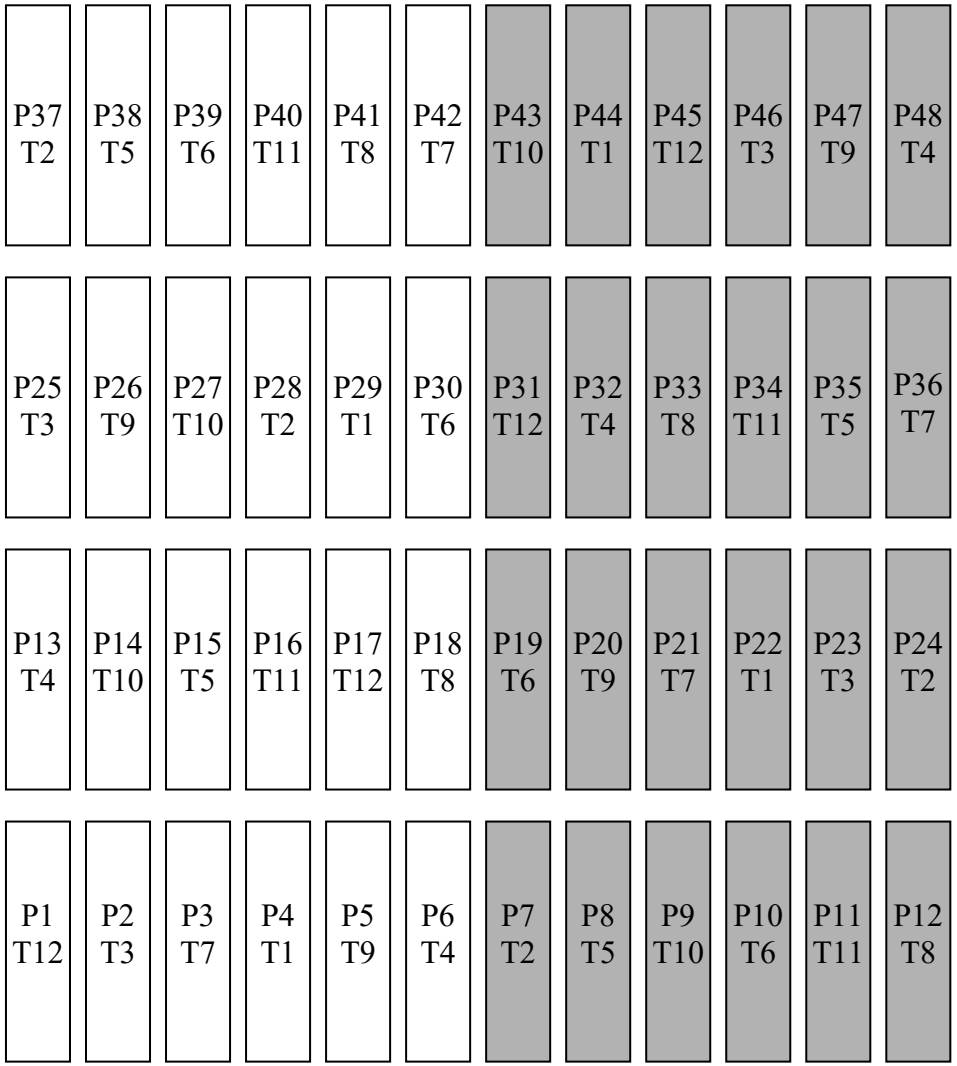


Treatments
1. Untreated control
1. Dithane 945
2. Fubol Gold
3. SL567A
4. Invader
5. Ranman Twinpack
6. Amistar
7. Shirlan
8. Epok
9. DP98
10. Programme 1
11. Programme 2

Appendix 1b : Trial Plan Site 2 Fungicide Trial

RMD in Red Beet – 2004 Trials E145

Fungicide Trial Plan Moore's

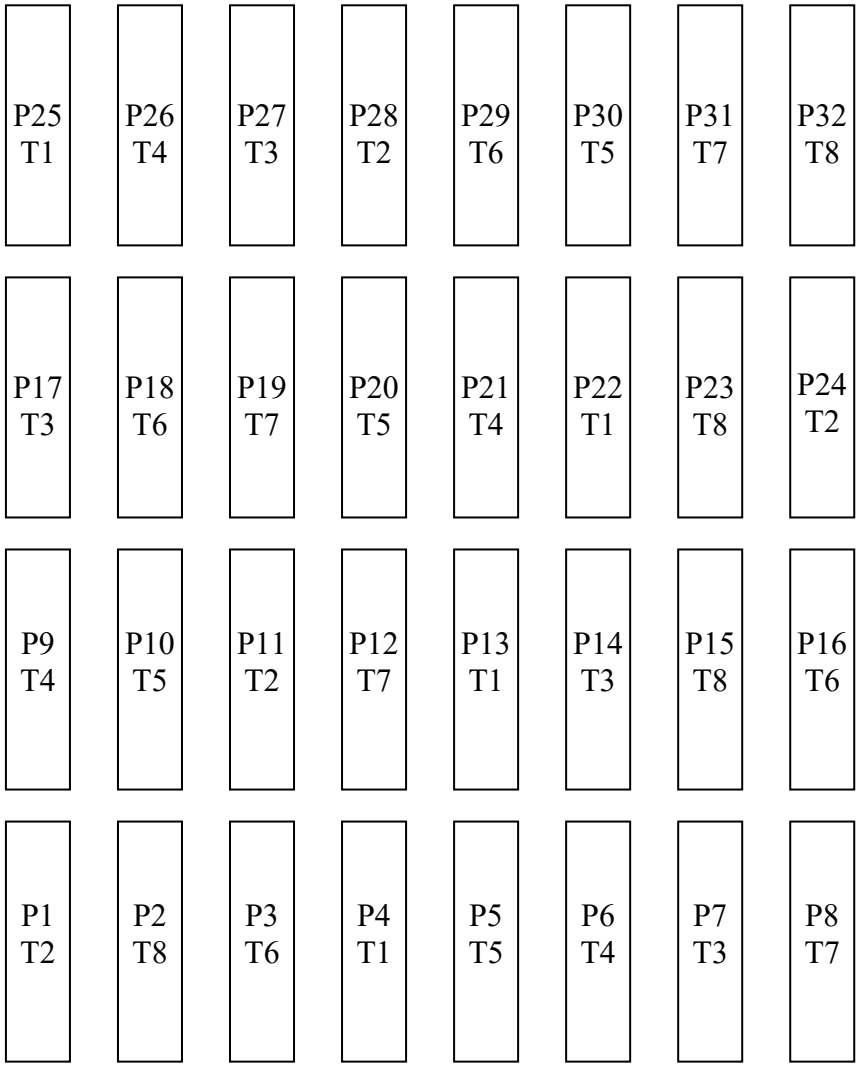


Treatments

1. Untreated control
2. Dithane 945
3. Fubol Gold
4. SL567A
5. Invader
6. Ranman Twinpack
7. Amistar
8. Shirlan
9. Epok
10. DP98
11. Programme 1
12. Programme 2

Wakil Treated Seed

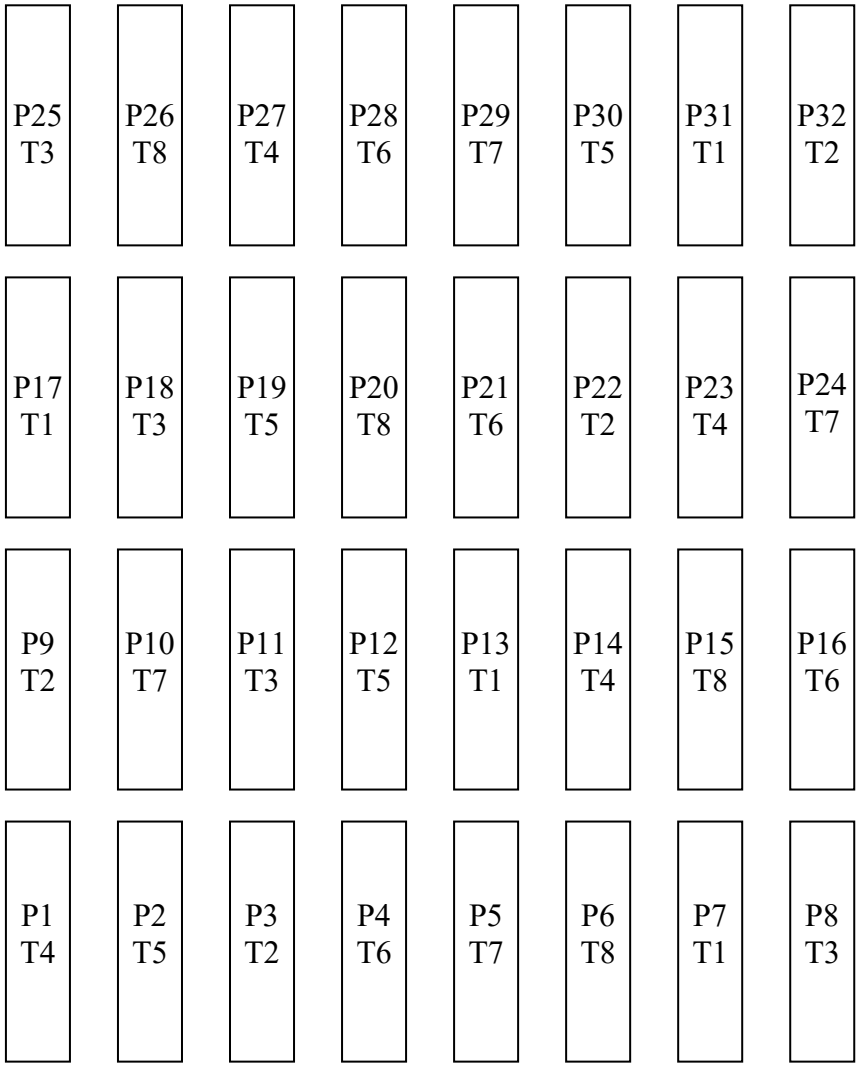
RMD in Red Beet – 2004 Trials E145
Application Timing Trial Plan Carr Farm



Treatment	June		July		Aug		Sept		Oct	
	1	2	1	2	1	2	1	2	1	2
1	+	+	+	+	+	+	+	+	+	+
2	+	+								
3			+	+						
4					+	+				
5							+	+		
6	+	+	+	+						
7				+	+	+	+			
8							+	+	+	+

All applications will be a tank mix of
Fubol Gold (1.9kg/ha) and Invader (2kg/ha)

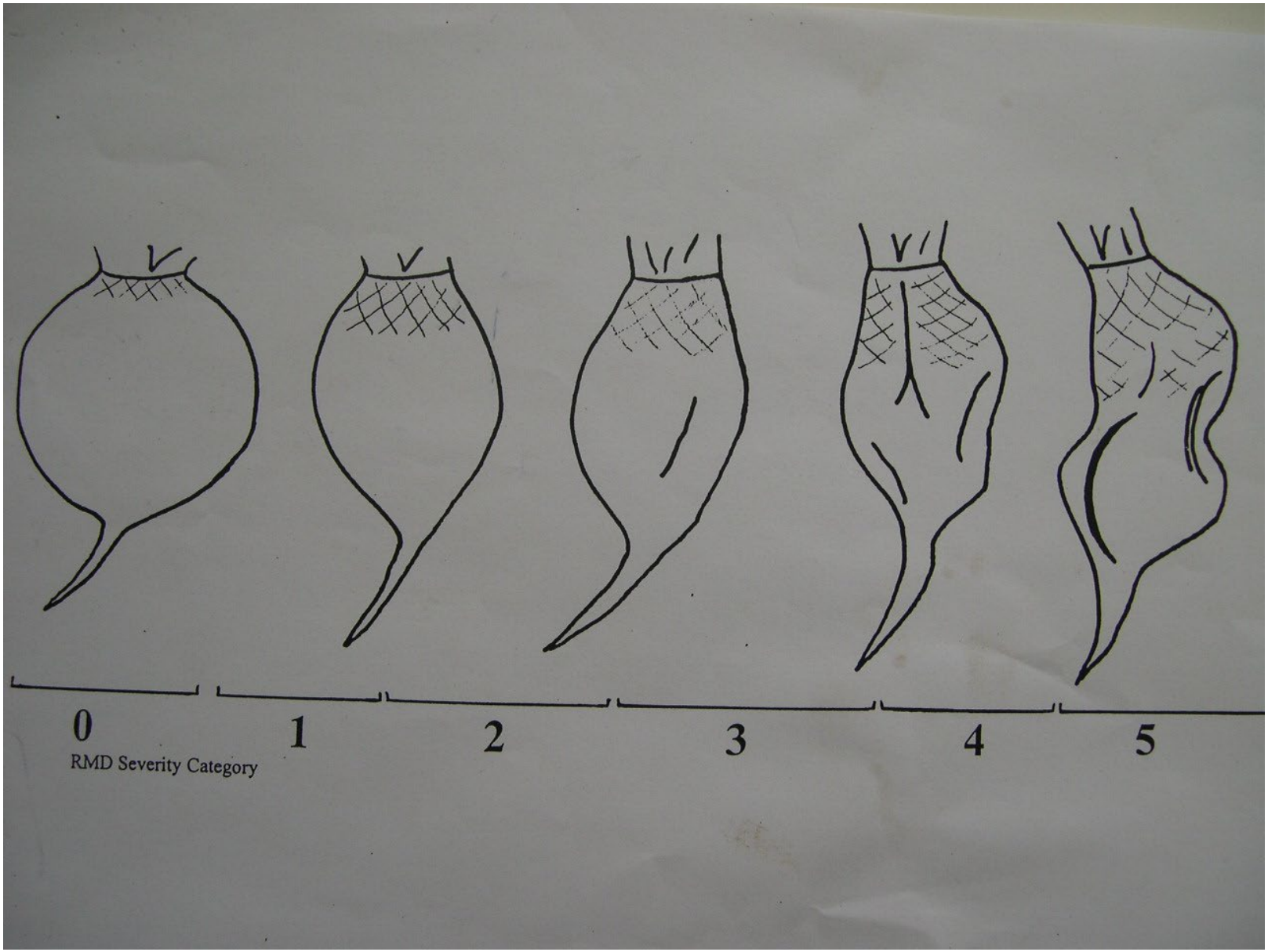
RMD in Red Beet – 2004 Trials E145
Application Timing Trial Plan Moore's Farm



Treatment	June		July		Aug		Sept		Oct	
	1	2	1	2	1	2	1	2	1	2
1	+	+	+	+	+	+	+	+	+	+
2	+	+								
3			+	+						
4					+	+				
5							+	+		
6	+	+	+	+						
7				+	+	+	+			
8							+	+	+	+

All applications will be a tank mix of
Fubol Gold (1.9kg/ha) and Invader (2kg/ha)

Appendix 3- Assessment scale for RMD symptoms



Appendix 4 – Results of quantitation of *P. farinosa* in Red beet field samples (CSL)

Sample Description	Quantity of *P. farinosa* per beet relative to positive control
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	0
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	0
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	0
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	7.82E-02
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	1.12E-03
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	0
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	2.62E-04
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	0
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	0
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	0
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	0
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	4.58E-02
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	1.99E-01
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	0
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	4.86E-03
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	0
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	no DNA
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	no DNA
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	0
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	no DNA
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	0
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	0
Arrived 11/06/04 small seedlings"Non Wakil trial guards"	3.60E-02
Healthy plant no mildew or other symptoms	1.31E-01
Healthy plant no mildew or other symptoms	1.61E-02
Healthy plant no mildew or other symptoms	2.68E-02
Healthy plant no mildew or other symptoms	8.31E-03
Healthy plant no mildew or other symptoms	3.11E-02
Healthy plant no mildew or other symptoms	4.63E-03
Healthy plant no mildew or other symptoms	1.44E-02
Healthy plant no mildew or other symptoms	1.53E-01
Healthy plant no mildew or other symptoms	1.49E-02
Healthy plant no mildew or other symptoms	3.23E-02
Healthy plant no mildew or other symptoms	5.23E-03
Healthy plant no mildew or other symptoms	4.83E-02
Distorted foliage no sporulation	2.08E+00
Distorted foliage no sporulation	3.26E+01
Distorted foliage no sporulation	1.82E+01
Distorted foliage no sporulation	7.97E-01
Distorted foliage no sporulation	5.05E-02
Distorted foliage no sporulation	1.04E+01
Distorted foliage no sporulation	6.45E-01
Distorted foliage no sporulation	3.91E-01
Distorted foliage no sporulation	4.56E+01
Distorted foliage no sporulation	7.18E+01

Distorted foliage no sporulation	2.42E+02
Distorted foliage no sporulation	1.94E+03
Distorted plants with sporulation	1.95E+01
Distorted plants with sporulation	7.75E+01
Distorted plants with sporulation	4.23E+02
Distorted plants with sporulation	2.33E+02
Distorted plants with sporulation	2.59E-01
Distorted plants with sporulation	5.51E+01
Distorted plants with sporulation	3.14E+02
Distorted plants with sporulation	2.18E+01
Distorted plants with sporulation	9.13E+01
Distorted plants with sporulation	3.02E+01
Distorted plants with sporulation	5.69E+01
Distorted plants with sporulation	3.21E+01
Moores Trials sites 24/6/04 P22 T1	6.27E-05
Moores Trials sites 24/6/04 P22 T1	3.31E-05
Moores Trials sites 24/6/04 P22 T1	1.64E-03
Moores Trials sites 24/6/04 P29 T1	1.25E-03
Moores Trials sites 24/6/04 P29 T1	7.63E-04
Moores Trials sites 24/6/04 P29 T1	6.36E-04
Moores Trials sites 24/6/04 P29 T1	7.70E-04
Moores Trials sites 24/6/04 P29 T1	2.83E-03
Moores Trials sites 24/6/04 P44 T1	2.95E-04
Moores Trials sites 24/6/04 P44 T1	2.17E-03
Moores Trials sites 24/6/04 P44 T1	3.71E-03
Moores Trials sites 24/6/04 P4 T1	0
Moores Trials sites 24/6/04 P4 T1	0
Moores Trials sites 24/6/04 P4 T1	4.99E-03
Moores Trials sites 24/6/04 P4 T1	8.75E-04
Crown Downy Mildew Beet 1. 22/6 arrived 24/6	2.91E+03
Crown Downy Mildew Beet 2. 22/6 arrived 24/6	6.19E+01
Crown Downy Mildew Beet 3. 22/6 arrived 24/6	6.32E+00
Crown Downy Mildew Beet 4. 22/6 arrived 24/6	2.20E+02
Crown Downy Mildew Beet 5. 22/6 arrived 24/6	4.95E+00
Crown Downy Mildew Beet 6. 22/6 arrived 24/6	9.09E+00
Crown Downy Mildew Beet 7. 22/6 arrived 24/6	2.71E+01
Crown Downy Mildew Beet 8. 22/6 arrived 24/6	5.11E+01
Crown Downy Mildew Beet 9. 22/6 arrived 24/6	9.29E+01
Crown Downy Mildew Beet 10. 22/6 arrived 24/6	4.13E+01
Healthy Beet 1 22/6 arrived 24/6	9.04E-03
Healthy Beet 2 22/6 arrived 24/6	1.32E+01
Healthy Beet 3 22/6 arrived 24/6	5.08E-02
Healthy Beet 4 22/6 arrived 24/6	1.75E-01
Healthy Beet 5 22/6 arrived 24/6	3.11E-02
Healthy Beet 6 22/6 arrived 24/6	3.48E-02
Healthy Beet 7 22/6 arrived 24/6	2.26E-01
Healthy Beet 8 22/6 arrived 24/6	1.47E+00
Healthy Beet 9 22/6 arrived 24/6	9.72E-02
Healthy Beet 10 22/6 arrived 24/6	5.08E-02
Distorted Beet 1 22/6 arrived 24/6	2.38E+01

Distorted Beet 2 22/6 arrived 24/6	1.04E-01
Distorted Beet 3 22/6 arrived 24/6	8.60E+02
Distorted Beet 4 22/6 arrived 24/6	2.16E+01
Distorted Beet 5 22/6 arrived 24/6	2.00E+03
Distorted Beet 6 22/6 arrived 24/6	1.26E+02
Distorted Beet 7 22/6 arrived 24/6	4.90E+01
Distorted Beet 8 22/6 arrived 24/6	7.12E-01
Distorted Beet 9 22/6 arrived 24/6	1.94E-01
Distorted Beet 10 22/6 arrived 24/6	1.13E+00
S Carr P7 T1 arrived 28/6/04 trial	1.13E-03
S Carr P7 T1 arrived 28/6/04 trial	2.53E-04
S Carr P7 T1 arrived 28/6/04 trial	7.52E-04
S Carr P41 T1 arrived 28/6/04 trial	2.24E-04
S Carr P41 T1 arrived 28/6/04 trial	1.28E-04
S Carr P41 T1 arrived 28/6/04 trial	0
S Carr P41 T1 arrived 28/6/04 trial	5.88E-04
S Carr P41 T1 arrived 28/6/04 trial	3.43E-03
S Carr P41 T1 arrived 28/6/04 trial	3.06E-04
S Carr P41 T1 arrived 28/6/04 trial	8.98E-04
S Carr P35 T1 arrived 28/6/04 trial	2.26E-04
S Carr P35 T1 arrived 28/6/04 trial	6.27E-03
S Carr P35 T1 arrived 28/6/04 trial	6.48E-04
S Carr P35 T1 arrived 28/6/04 trial	4.40E-03
S Carr P35 T1 arrived 28/6/04 trial	8.51E-04
S Carr P35 T1 arrived 28/6/04 trial	no DNA
S Carr P15 T1 arrived 28/6/04 trial	0
S Carr P15 T1 arrived 28/6/04 trial	2.88E-03
S Carr P15 T1 arrived 28/6/04 trial	0
P29 West Butterwick arrived 7/7/04	0
P29 West Butterwick arrived 7/7/04	6.80E-05
P29 West Butterwick arrived 7/7/04	1.63E-02
P22 West Buterwick arrived 7/7/04	0
P22 West Buterwick arrived 7/7/04	0
P22 West Buterwick arrived 7/7/04	0
P4 West Butterwick arrived 7/7/04	3.97E-04
P4 West Butterwick arrived 7/7/04	0
P4 West Butterwick arrived 7/7/04	0
P41 South Carr Farm arrived 7/7/04	3.11E-04
P41 South Carr Farm arrived 7/7/04	3.70E+01
P35 South Carr farm arrived 7/7/04	7.82E-04
P35 South Carr farm arrived 7/7/04	1.71E-04
PH yellow tags 0/m infected crown arrived 7/7/04	9.73E-01
PH Green Canes 'healthy' arrived 7/7/04	1.21E+00
P7 South Carr Farm arrived 7/7/04	9.03E-05
P7 South Carr Farm arrived 7/7/04	3.52E+00
P15 South Carr Farm arrived 7/7/04	0
P15 South Carr Farm arrived 7/7/04	4.48E-04
P44 West Butterwick arrived 7/7/04	0
P44 West Butterwick arrived 7/7/04	2.63E-04
PH White tags arrived 7/7/04	6.08E+00

White Tag arrived 26/7/04	1.57E+00
White Tag arrived 26/7/04	6.44E+00
White Tag arrived 26/7/04	0
White Tag arrived 26/7/04	7.05E-01
White Tag arrived 26/7/04	2.01E+01
Yellow Tag arrived 26/7/04	3.23E+03
Yellow Tag arrived 26/7/04	2.52E+01
Yellow Tag arrived 26/7/04	2.08E+01
Yellow Tag arrived 26/7/04	1.45E+02
Yellow Tag arrived 26/7/04	8.35E-03
Green Canes arrived 26/7/04	no DNA
Green Canes arrived 26/7/04	1.45E-02
Green Canes arrived 26/7/04	0
Green Canes arrived 26/7/04	3.70E-02
Green Canes arrived 26/7/04	no DNA
West Butterwick P44 arrived 26/7/04	8.69E-04
West Butterwick P44 arrived 26/7/04	5.46E-04
West Butterwick P44 arrived 26/7/04	4.65E-04
West Butterwick P4 arrived 26/7/04	0
West Butterwick P4 arrived 26/7/04	0
West Butterwick P4 arrived 26/7/04	0
West Butterwick P22 arrived 26/7/04	2.30E-04
West Butterwick P22 arrived 26/7/04	0
West Butterwick P22 arrived 26/7/04	0
West Butterwick P29 arrived 26/7/04	0
West Butterwick P29 arrived 26/7/04	0
West Butterwick P29 arrived 26/7/04	0
South Carr P15 arrived 26/7/04	1.41E-03
South Carr P15 arrived 26/7/04	1.16E-04
South Carr P15 arrived 26/7/04	7.32E-03
South Carr P7 arrived 26/7/04	6.84E-04
South Carr P7 arrived 26/7/04	1.43E-04
South Carr P7 arrived 26/7/04	2.45E-04
South Carr P35 arrived 26/7/04	5.74E-05
South Carr P35 arrived 26/7/04	2.74E-03
South Carr P35 arrived 26/7/04	0
South Carr P41 arrived 26/7/04	0
South Carr P41 arrived 26/7/04	0
South Carr P41 arrived 26/7/04	0
Green Canes arrived 4/8/04	0
White Labels arrived 4/8/04	no DNA
Yellow Labels arrived 4/8/04	no DNA
SC P15 arrived 4/8/04	0
SC P15 arrived 4/8/04	1.09E+01
SC P7 arrived 4/8/04	9.61E+00
SC P7 arrived 4/8/04	0
SC P35 arrived 4/8/04	1.49E+01
SC P35 arrived 4/8/04	0
SC P41 arrived 4/8/04	3.05E+00
SC P41 arrived 4/8/04	6.16E-04

WB P22 arrived 4/8/04	0
WB P22 arrived 4/8/04	5.58E+00
WB P4 arrived 4/8/04	6.87E-04
WB P4 arrived 4/8/04	0
WB P29 arrived 4/8/04	0
WB P29 arrived 4/8/04	0
WB P44 arrived 4/8/04	0
WB P44 arrived 4/8/04	0
SC P35 T1 arrived 17/8/04	0
SC P35 T1 arrived 17/8/04	5.81E+00
SC P41 T1 arrived 17/8/04	0
SC P41 T1 arrived 17/8/04	1.47E+00
SC P41 T1 arrived 17/8/04	7.93E+01
SC P15 T1 arrived 17/8/04	0
SC P15 T1 arrived 17/8/04	1.21E+01
SC P7 T1 arrived 17/8/04	5.81E-01
SC P7 T1 arrived 17/8/04	7.04E+00
Green arrived 17/8/04	3.84E-01
Green arrived 17/8/04	0
Green arrived 17/8/04	0
Green arrived 17/8/04	0
Green arrived 17/8/04	0
Yellow arrived 17/8/04	4.65E+00
Yellow arrived 17/8/04	7.92E+01
Yellow arrived 17/8/04	0
Yellow arrived 17/8/04	0
Yellow arrived 17/8/04	9.55E-01
White arrived 17/8/04	0
White arrived 17/8/04	0
White arrived 17/8/04	0
White arrived 17/8/04	0
White arrived 17/8/04	1.28E+01
Cooper 16/8/04 1H Healthy arrived 17/8/04	0
Cooper 16/8/04 2H Healthy arrived 17/8/04	0
Cooper 16/8/04 3H Healthy arrived 17/8/04	0
Cooper 16/8/04 4H Healthy arrived 17/8/04	0
Cooper 16/8/04 5H Healthy arrived 17/8/04	0
Cooper 16/8/04 6H Healthy arrived 17/8/04	0
Cooper 16/8/04 7H Healthy arrived 17/8/04	0
Cooper 16/8/04 8H Healthy arrived 17/8/04	0
Cooper 16/8/04 9H Healthy arrived 17/8/04	0
Cooper 16/8/04 10H Healthy arrived 17/8/04	0
Cooper 16/8/04 1A Affected arrived 17/8/04	0
Cooper 16/8/04 2A Affected arrived 17/8/04	1.36E+01
Cooper 16/8/04 3A Affected arrived 17/8/04	7.82E+00
Cooper 16/8/04 4A Affected arrived 17/8/04	0
Cooper 16/8/04 5A Affected arrived 17/8/04	2.80E+01
Cooper 16/8/04 6A Affected arrived 17/8/04	5.21E-03
Cooper 16/8/04 7A Affected arrived 17/8/04	0
Cooper 16/8/04 8A Affected arrived 17/8/04	3.90E+01

Cooper 16/8/04 9A Affected arrived 17/8/04	7.08E+00
Cooper 16/8/04 10A Affected arrived 17/8/04	5.28E+01
Cooper 16/8/04 11A Affected arrived 17/8/04	4.69E+01
Cooper 16/8/04 12A Affected arrived 17/8/04	1.74E+01
Cooper 16/8/04 13A Affected arrived 17/8/04	5.09E+01
SC P41 arrived 1/9/04	3.74E+00
SC P41 arrived 1/9/04	0
SC P35 arrived 1/9/04	0
SC P35 arrived 1/9/04	0
SC P15 arrived 1/9/04	8.37E-01
SC P15 arrived 1/9/04	2.11E+00
SC P7 arrived 1/9/04	5.63E+00
SC P7 arrived 1/9/04	3.58E+01
Yellow 6/9/04	no DNA
Yellow 6/9/04	0
Yellow 6/9/04	0
Yellow 6/9/04	3.52E+04
Yellow 6/9/04	1.38E+00
White 6/9/04	0
White 6/9/04	6.71E-01
White 6/9/04	4.01E+01
White 6/9/04	0
White 6/9/04	0
Green 6/9/04	0
Green 6/9/04	0
Green 6/9/04	7.02E-01
Green 6/9/04	0
Green 6/9/04	0
S.carr P41 24/9/04 arrived 27/9/04	9.34E-02
S.carr P41 24/9/04 arrived 27/9/04	0
S.carr P7 24/9/04 arrived 27/9/04	0
S.carr P7 24/9/04 arrived 27/9/04	1.25E-03
S.carr 351 24/9/04 arrived 27/9/04	6.62E-05
S.carr P35 24/9/04 arrived 27/9/04	7.02E-01
S.carr P15 24/9/04 arrived 27/9/04	5.27E-02
S.carr P15 24/9/04 arrived 27/9/04	1.02E-03
Yellow Tag 1 24/9/04 arrived 27/9/04	0
Yellow Tag 2 24/9/04 arrived 27/9/04	6.30E-01
Yellow Tag 3 24/9/04 arrived 27/9/04	0
Yellow Tag 4 24/9/04 arrived 27/9/04	9.43E-02
Yellow Tag 5 24/9/04 arrived 27/9/04	1.40E-01
White Tag 1 24/9/04 arrived 27/9/04	0
White Tag 2 24/9/04 arrived 27/9/04	0
White Tag 3 24/9/04 arrived 27/9/04	0
White Tag 4 24/9/04 arrived 27/9/04	7.14E+00
White Tag 5 24/9/04 arrived 27/9/04	0
Green Peas 1 24/9/04 arrived 27/9/04	2.56E+00
Green Peas 2 24/9/04 arrived 27/9/04	0
Green Peas 3 24/9/04 arrived 27/9/04	0
Green Peas 4 24/9/04 arrived 27/9/04	0

Green Peas 5 24/9/04 arrived 27/9/04	0
Green Peas 6 24/9/04 arrived 27/9/04	3.56E+01
Green Peas 7 24/9/04 arrived 27/9/04	0
Green Peas Healthy 8 24/9/04 arrived 27/9/04	0
Green Peas 9 24/9/04 arrived 27/9/04	0
Green Peas 10 24/9/04 arrived 27/9/04	7.94E-01
White Tag 22/10/04 arrived 25/10/04	9.80E+00
White Tag 22/10/04 arrived 25/10/04	7.55E+01
White Tag 22/10/04 arrived 25/10/04	3.03E-02
White Tag 22/10/04 arrived 25/10/04	0
White Tag 22/10/04 arrived 25/10/04	4.97E-02
White Tag 22/10/04 arrived 25/10/04	2.55E-03
White Tag 22/10/04 arrived 25/10/04	no DNA
White Tag 22/10/04 arrived 25/10/04	1.65E+00
White Tag 22/10/04 arrived 25/10/04	3.06E+01
White Tag 22/10/04 arrived 25/10/04	0
White Tag 22/10/04 arrived 25/10/04	0
White Tag 22/10/04 arrived 25/10/04	1.30E+02
White Tag 22/10/04 arrived 25/10/04	5.38E-03
White Tag 22/10/04 arrived 25/10/04	5.16E+00
White Tag 22/10/04 arrived 25/10/04	0
White Tag 22/10/04 arrived 25/10/04	0
White Tag 22/10/04 arrived 25/10/04	4.65E-01
White Tag 22/10/04 arrived 25/10/04	0
White Tag 22/10/04 arrived 25/10/04	2.53E+00
White Tag 22/10/04 arrived 25/10/04	0
White Tag 22/10/04 arrived 25/10/04	3.39E+00
White Tag 22/10/04 arrived 25/10/04	1.53E-01
White Tag 22/10/04 arrived 25/10/04	0
White Tag 22/10/04 arrived 25/10/04	1.64E+01
White Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	3.22E+00
Yellow Tag 22/10/04 arrived 25/10/04	4.53E-01
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	1.05E+00
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	2.89E-01
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	3.10E+01
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	1.09E+01
Yellow Tag 22/10/04 arrived 25/10/04	0

Yellow Tag 22/10/04 arrived 25/10/04	5.21E-01
Yellow Tag 22/10/04 arrived 25/10/04	2.71E-01
Yellow Tag 22/10/04 arrived 25/10/04	8.35E-01
Yellow Tag 22/10/04 arrived 25/10/04	2.01E+00
Yellow Tag 22/10/04 arrived 25/10/04	3.61E+00
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	4.63E+00
Yellow Tag 22/10/04 arrived 25/10/04	7.61E-03
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	1.49E+01
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	2.92E-02
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	4.19E+00
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	no DNA
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	1.90E+00
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	1.49E+00
Yellow Tag 22/10/04 arrived 25/10/04	1.13E+00
Yellow Tag 22/10/04 arrived 25/10/04	5.99E+00
Yellow Tag 22/10/04 arrived 25/10/04	4.21E+00
Yellow Tag 22/10/04 arrived 25/10/04	2.28E-02
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	2.19E+03
Yellow Tag 22/10/04 arrived 25/10/04	1.01E+01
Yellow Tag 22/10/04 arrived 25/10/04	no DNA
Yellow Tag 22/10/04 arrived 25/10/04	2.34E+01
Yellow Tag 22/10/04 arrived 25/10/04	1.14E+00
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	2.02E+02
Yellow Tag 22/10/04 arrived 25/10/04	0
Yellow Tag 22/10/04 arrived 25/10/04	5.73E-01
Yellow Tag 22/10/04 arrived 25/10/04	1.13E-01
Yellow Tag 22/10/04 arrived 25/10/04	7.74E+02
Green Tag 22/10/04 arrived 25/10/04	0

Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	6.81E-03
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	5.73E-03
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	9.26E-02
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	3.22E-03
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	1.27E-01
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	3.98E+02
Green Tag 22/10/04 arrived 25/10/04	1.99E-01
Green Tag 22/10/04 arrived 25/10/04	1.13E-02
Green Tag 22/10/04 arrived 25/10/04	5.24E-03
Green Tag 22/10/04 arrived 25/10/04	1.49E+02
Green Tag 22/10/04 arrived 25/10/04	7.83E-02
Green Tag 22/10/04 arrived 25/10/04	5.66E-02
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	5.23E+00
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0

Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	2.76E+00
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	0
Green Tag 22/10/04 arrived 25/10/04	8.69E+01
Green Tag 22/10/04 arrived 25/10/04	0
Control Grab Sample 22/10/04 arrived 25/10/04	1.16E+02
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	8.11E-03
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	1.84E+00
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	2.43E-02
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	0
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	0
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	0
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	0
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	0
Control Grab Sample 22/10/04 arrived 25/10/04	0
Control Grab Sample 22/10/04 arrived 25/10/04	3.95E-03
Control Grab Sample 22/10/04 arrived 25/10/04	0
Control Grab Sample 22/10/04 arrived 25/10/04	0
Control Grab Sample 22/10/04 arrived 25/10/04	0
Control Grab Sample 22/10/04 arrived 25/10/04	0
Control Grab Sample 22/10/04 arrived 25/10/04	0
Control Grab Sample 22/10/04 arrived 25/10/04	0
Control Grab Sample 22/10/04 arrived 25/10/04	0
Control Grab Sample 22/10/04 arrived 25/10/04	0
Control Grab Sample 22/10/04 arrived 25/10/04	1.43E-02
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	3.16E-03
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	0
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	0
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	no DNA
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	8.39E+00
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	1.59E+01
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	1.24E-02
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	5.91E+00
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	5.70E+00
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	3.73E+01
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	2.26E+02
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	0
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	0
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	2.88E+00
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	1.28E+02
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	1.54E-02
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	0
Control Grab Sample (Distorted roots) 22/10/04 arrived 25/10/04	3.84E-01
Plot 38; Healthy roots arrived 8/11/04	0
Plot 34; Healthy roots arrived 8/11/04	0

Plot 15; Healthy roots arrived 8/11/04	0
Plot 6; Healthy roots arrived 8/11/04	5.92E-04
Plot 9; Healthy roots arrived 8/11/04	1.73E+02
Plot 23; Healthy roots arrived 8/11/04	5.84E+01
Plot 7; Healthy roots arrived 8/11/04	1.96E+00
Plot 22; Healthy roots arrived 8/11/04	0
Plot 26; Healthy roots arrived 8/11/04	1.22E-02
Plot 16; Healthy roots arrived 8/11/04	1.09E-02
Plot 45; Healthy roots arrived 8/11/04	1.50E-03
Plot 41; Healthy roots arrived 8/11/04	0
Plot 35; Healthy roots arrived 8/11/04	5.30E+00
Plot 19; Healthy roots arrived 8/11/04	0
Plot 42; Healthy roots arrived 8/11/04	3.03E-01
Plot 44; Healthy roots arrived 8/11/04	0
Plot 45; Affected roots arrived 8/11/04	1.39E+01
Plot 42; Affected roots arrived 8/11/04	6.55E+02
Plot 34; Affected roots arrived 8/11/04	0
Plot 7; Affected roots arrived 8/11/04	4.89E+01
Plot 44; Affected roots arrived 8/11/04	9.44E-03
Plot 16; Affected roots arrived 8/11/04	2.28E+00
Plot 41; Affected roots arrived 8/11/04	0
Plot 38; Affected roots arrived 8/11/04	0
Plot 26; Affected roots arrived 8/11/04	9.67E-02
Plot 35; Affected roots arrived 8/11/04	0
Plot 23; Affected roots arrived 8/11/04	1.76E+00
Plot 15; Affected roots arrived 8/11/04	7.20E+02
Plot 9; Affected roots arrived 8/11/04	6.80E-02
Plot 19; Affected roots arrived 8/1//044	no DNA
Plot 22; Affected roots arrived 8/11/04	1.31E-03
Plot 6; Affected Roots arrived 8/11/04	2.33E-01