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

Project Number: FV 226a

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Report: Final Report, July 2004

Location: STC Ltd and grower crop trials in South Yorkshire

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Date Commenced: April 2003

Completion date: March 2004

Key Words: Red beet, beetroot, root distortion, malformation, RMD, downy mildew, *Peronospora farinosa*, *Rhizoctonia*, *Pythium* spp., disease control, fungicides, crop safety, efficacy, soil sterilants, pesticide, fungicide, disease, SL 567A, metalaxyl-M, Amistar, azoxystrobin, Fubol Gold, metalaxyl, mancozeb, Basilex, tolclofos-methyl, Bavistin, carbendazim, Biomex, Trichoderma, Vitomex, phosphite.

The results and conclusions in this report are based on a series of 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.

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FV 226a : GROWER SUMMARY

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

Headlines

- A strong correlation between the presence of downy mildew, the occurrence of a brown petiole symptom in plants and root malformation was found in some commercial crops and in the trial at Site 2.

- At the Westwoodside site there was a very strong correlation between crop vigour at the end of the season and the applied fungicides. This was attributed to the control of foliar disease, primarily rust (*Uromyces betae*), at this site with Amistar proving to be the most effective product. The dithiocarbamate (mancozeb) component of Fubol Gold and Invader also proved surprisingly effective. Bavistin gave a moderate suppression of the rust disease, whereas SL567A, Basilex and Biomex/Vitomex were ineffective.

- An extensive literature search has indicated that the downy mildew (*P. farinosa* f. sp. *chenopodii*) on Chenopodiaceous weeds such as 'fat-hen' (*Chenopodium album*), is different to that (*P. farinosa* f. sp. *spinaciae*) which occurs on Spinach (*Spinacia oleracea*) and this in turn is different to that (*P. farinosa* f. sp. *betae*) which occurs on commercial red beet or sugar beet (*Beta vulgaris*). Cross-inoculation studies between the different host-pathogen combinations, as reported in the scientific literature, have proved negative and therefore it must be concluded that the downy mildew inoculum pressure from 'fat-hen' or related weeds presents no infection risk to the red beet crop. However, any infection in commercial sugar-beet crops is likely to provide inoculum for potential cross-infection to red beet crops in the vicinity.

- In parallel with this trials work, SOLAs have been secured for; Amistar, Filex, SL 567A and Wakil XL, which will help maintain crop health and disease control.

- Tests developed at CSL have given an extremely clear positive result for *P. farinosa* in each case using a batch of 10 individual RMD affected roots and a clear negative for 10 non-RMD affected roots. This provides the clearest evidence yet that systemic infection with *P. farinosa* is responsible for RMD.

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 evaluate 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. A web-based report from Oregon in the USA describes symptoms of d. mildew in red beet (Plate 4) that correlates closely with those of RMD.

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

The primary aim of the project in 2003 project was 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. Separately, a search of past scientific literature on the subject was conducted. The primary aim 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.

Separately, and towards the end of the project in 2003-2004 HDC established requested CSL to develop a novel molecular method for DNA analysis of affected and unaffected roots as a means of determining conclusively whether the downy mildew pathogen was implicated in the disorder.

Summary of the Project and Main Conclusions

(i) Replicated trial sites with fungicide treatments

Following discussion with industry representatives two sites for trial purposes were identified on commercial farms in South Yorkshire. At each site half the area was treated with the soil sterilant product metham sodium (Discovery) by Sands Agricultural Services Ltd (now Countrywide Farmers) during late April 2003. Red beet seed cultivars Darko (Site 1 – Westwoodside) & Crimson Globe (Site 2 – West Butterwick) were drilled in early May and a range of fungicide and related treatments applied on a replicated basis almost immediately. All sprays were applied using purpose-designed tractor-mounted equipment. Spray treatments were applied at approximate 4 week intervals aiming to provide broad protection from drilling through to maturity.

Trial Site 1 (Westwoodside)

At this site seedling establishment was relatively poor, especially in some low lying 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 all the affected seedlings and was considered to be the primary cause for the establishment problems at this site. Perhaps not surprisingly there was a significant difference in this regard between the sterilised and unsterilised plots. Unfortunately, none of the individual applied chemical treatments in the trial provided complete control of the disease though some may have given a slight reduction in disease severity.

Because of the high drilling density at this site sufficient plants survived to justify taking the trial through to crop maturity. Whilst low levels of downy mildew developed on the foliage/crown tissues of occasional plants in the trial area by July few RMD symptoms could be found and where present the symptoms were very mild and this could have been caused by other factors. By crop maturity in October-November there was a negligible level of RMD in any of the trial plots. Apart from a general effect in overall plant vigour (due largely to the impact of the *Aphanomyces*) the only other visible effect in the crop during establishment was a marked reduction in weed growth in the sterilised area compared with the non-sterilised area. However, by late November some plots appeared to remain more vigorous with strong top growth compared to other less vigorous plots where the foliage had died back following early frosts.

Interestingly, an assessment of plot vigour made on 3 December highlighted a strong correlation between the applied treatments with Amistar, Fubol Gold, Invader and, to a lesser extent, Bavistin providing significantly improved crop vigour. Close inspection of the disease assessment data also shows a strong correlation with rust (*Uromyces betae*) in the trial crop and this almost certainly accounts for the improved foliage vigour late in the crop. In the case of Fubol Gold and Invader it is considered that the dithiocarbamate (mancozeb) component of the fungicide mixture is likely to have provided considerable protectant activity against this disease.

Due to the lack of a significant development of RMD in this trial site and the absence of downy mildew or any other potential cause for root malformation no further assessments were conducted though the site will be retained *in situ* over-winter.

Trial Site 2 (West Butterwick)

At this site seedling germination was slower than at Site 1 and this was due in part to the relatively wet heavy land used for the site and emergence occurred over a longer time period. Ultimately, plant density at this site was much lower though it did not suffer any apparent problems with *Aphanomyces cochlioides*. Downy mildew was found on occasional plants in the trial site on 23 July and continued at low-moderate levels as the season progressed. Surprisingly, this infection appeared, during routine visual inspection, to be present irrespective of any of the applied fungicides. Plants with RMD

symptoms were also observed on 23 July and a detailed assessment *in situ* at this early stage in the trial hinted at a possible correlation between the presence of this obligate pathogen and the occurrence of RMD on the same 'infected' plants.

However, none of the applied oomycete fungicides completely eliminated downy mildew from the trial plots during the season, even after 4-5 fungicide applications. Treatment with metalaxyl & metalaxyl+mancozeb proved largely ineffective though resistance to metalaxyl could account for its poor performance. Interestingly though, Invader, another oomycete fungicide with a different mode of action, was also moderately effective. Amistar, a broad-spectrum protectant fungicide with moderate activity against d. mildew fungi also provided a reasonable suppression of d. mildew at this Autumn assessment. Products with no activity against d. mildew (eg Bavistin, Basilex) were largely ineffective in preventing RMD at this site. Whilst this provides yet further evidence to support the hypothesis that an oomycete fungus such as d. mildew may be implicated in RMD the primary cause continues to be open to some speculation.

In final assessments in November downy mildew could still be found at relatively low levels in most plots. In the unsterilised area Invader appeared very effective against both d. mildew & RMD. However, a similar result was not achieved in the sterilised trial area and this variability makes interpretation of the trial data very difficult. Generally, the incidence of d. mildew and RMD was reduced in the sterilised area, as compared to the equivalent unsterilised plots, though was not eliminated completely. This suggests that there may be a soil-borne phase to the disorder though, at the same time, also indicates that there may be other inoculum sources which has allowed the problem to occur even in sterilised plots.

An improved plant vigour was also noted at this site in late Autumn though this appeared to be unrelated to the presence of leaf disease in the crop. Unlike at Site 1, there was little rust at this site and instead *Cercospora* leaf-spot predominated. This appeared not to be well controlled with any of the applied fungicides and there appeared to be little or no correlation between this disease and plant vigour in late November 2003. The improved plant vigour observed in certain plots at site 2 cannot therefore be accounted for at this stage.

Yield data collected at this site indicated that there had been little impact of the various fungicide treatments on the total bulk weight of the crop. Even the soil sterilisation treatment appeared to have little effect in this regard.

In summary, at this trial site downy mildew was the only recognised pathogen to occur at appreciable levels, though it was not particularly well controlled by the various applied fungicides, and this is particularly surprising and disappointing. It may be that the 4 week interval between applications was insufficient and a shorter time between sprays may be required in future. RMD did occur in this trial crop though was somewhat sporadic and variable in its occurrence. In the early stages of the trial there appeared to be a good correlation between d. mildew infected plants and the development of RMD though this effect appeared to become less clear as the season progressed. By the end of the trial it was evident that RMD symptoms had been suppressed to a limited extent following soil sterilisation and this implies a possible soil-borne phase to the problem. Application of the various fungicides gave mixed results which cannot be readily explained, though this may relate to the relatively poor control of d. mildew.

(ii) The role of weed hosts for downy mildew development

Downy mildew has also been observed at high levels in recent years on *Chenopodium album* or 'fat-hen a common weed in UK agriculture, including in red beet crops. Whether this strain was the same as that infecting red beet remained uncertain at commencement of the work programme though it was considered that the increased acreage of 'set-aside' in the last 5 years could potentially have accounted for an upsurge in both the weed host and inoculum of this air-borne pathogen.

A detailed search of the scientific literature using an extensive series of key-words (downy mildew, *Peronospora farinosa*, *Peronospora schactii*, *Chenopodiaceae*, *Chenopodium album*, beet, beetroot, red beet, *Beta vulgaris*, sugar beet, goosefoot, fat-hen, cross-inoculation, spinach, *Spinacia oleracea*, chard, weeds, strain variation) has now demonstrated that they are in fact different strains of *P. farinosa* as follows:-

- *Peronospora farinosa* f. sp. *betae* on Red Beet & Sugar Beet
- *Peronospora farinosa* f. sp. *chenopodii* on Quinoa and other *Chenopodium* species, including *C. album* or 'fat-hen'
- *Peronospora farinosa* f. sp. *spinaciae* on Spinach (*Spinacia oleracea*)

The inference from this historic cross-inoculation work (references provided) is that the downy mildew (*P. farinosa* f.sp. *chenopodii*) observed on 'fat-hen' or goosefoot (*Chenopodium album*) in beet crops presents no risk to red beet or sugar beet crops in the vicinity as these are only susceptible to the host specialised form of the pathogen (*P. farinosa* f.sp. *betae*).

(iii) Other observations in Red Beet crops during 2003

During Autumn 2002 and during the 2003 cropping season it became evident during close examination of affected crops and in talking with industry representatives that other symptoms were, on occasions, associated with RMD. RMD affected plants frequently, though not exclusively, developed an orange-brown discoloration of the petioles. The symptom was considered very similar to the colour generated on beet leaves known to be systemically infected by downy mildew in the crown. It should be noted however that, in some cases, this brown petiole symptom may also be a varietal characteristic and therefore cannot be used alone to distinguish RMD affected plants in a crop.

In some crops, particularly during the dry summer conditions of 2003, it was possible to identify RMD affected roots by the erect nature of the central crown leaves. Whereas healthy plants were wilting due to a significant soil moisture deficit the central leaves of RMD affected plants remained erect. This may be due to the reduced sugar content of the leaves or possibly a response from the thicker tap root present on many of the RMD affected plants.

(a) Tagging plants

In one commercial crop inspected during late August 2003 the incidence of downy mildew was found to be relatively high. It was decided to 'tag' plants which showed 3 distinct symptoms:-

1. Healthy plants, no downy mildew or other symptoms visible
2. Crown infection with downy mildew, sporulation clearly evident
3. Brown petiole symptom, no sporulation of downy mildew

The plants in the 3 categories above were identified and tagged on 4 September and then the crop was left undisturbed until near harvest at which time the plants in the three categories were lifted and returned to the laboratory for detailed assessment. At the time the plants were lifted on 7 October there was a relatively high incidence of RMD. Many of the plants were wilting due to a high soil moisture deficit yet RMD affected plants were more prominent because they stood more erect in the crop.

Interestingly, on the plants which were visibly free of d. mildew infection at the time of tagging 39% developed RMD symptoms (Table 3 & Figure 9). This could either be due to latent infection not visible at the time of tagging or that they became infected later after the tagging had been completed. There was a much higher incidence of

RMD in plants which were visibly infected with d. mildew at the time of tagging and this is highly significant. Also, those plants with a brown petiole symptom also had a higher incidence of RMD, though interestingly in this study, the disease index (RMD severity) was also greatly increased.

It may be appropriate to undertake more of this type of 'tagged' monitoring in subsequent crops to further investigate a possible link between the various symptoms.

(b) Monitoring the 'brown petiole' symptom

In a separate observational study in a red beet crop which exhibited brown petiole symptoms in November 2003 we collected 10-15 healthy and 10-15 'brown petiole' plants from 2 random areas of the crop. We returned these to the laboratory forassessment of RMD and associated symptoms. What is particularly interesting is that there again appeared to be a strong correlation between the incidence of the 'brown petiole' symptom and RMD. Where healthy plants were randomly selected from this crop ie no 'brown petiole' evident, the incidence of RMD was zero. However, in contrast, in all cases where plants were selected which exhibited a 'brown petiole' symptom RMD was present on the root. Of even more significance was that we found a very low incidence (3.3%) of downy mildew on the plants with healthy petioles whereas on the plants with 'brown petiole' symptoms 68% of the plants exhibited d. mildew in the crown tissues.

Preliminary results for the various trials and observations during 2003 were mixed though there was considerable evidence for a possible link between systemic infection with d. mildew and RMD. It was particularly unfortunate (from an experimental basis) that 2003 proved to be unusual climatically and not conducive to the development of wet weather diseases such as d. mildew as this marred our chances of securing valuable data on fungicide performance against RMD in red beet.

(iv) Pin-pointing the cause of RMD

During January-March 2004 CSL scientists developed an assay for *Peronospora farinosa*. This new technique based on a PCR-Taqman assay works by detecting and amplifying a unique piece of the pathogens DNA profile. In this way it can be used to confirm the presence of a specific pathogen without the need to culture onto artificial growing media. After validation against this and other pathogens, it was used to check healthy and affected red beet for systemic infection with *P. farinosa*. In the initial test 10 healthy and 10 affected beet were taken randomly from a stored crop and processed using the PCR-Taqman assay.

The results from the PCR-Taqman study at CSL proved to be extremely enlightening. An initial test taking tissue from the crown region in each root showed 70% of the RMD affected roots to be systemically infected with *P. farinosa*. A subsequent study on individual beet demonstrated a higher recovery of DNA of *P. farinosa* in the lower section of the root. When the test was repeated on the same two lots of 10 roots 100% proved to be infected systemically with *P. farinosa*. None of the non-distorted roots yielded DNA of *P. farinosa*. Whilst it will be necessary to conduct further tests on affected & healthy roots during the 2004 this initial test, especially in conjunction with in-field observations, strongly suggests that RMD is caused by a systemic invasion by the d. mildew fungus *P. farinosa*. In light of this new information it becomes even more imperative to evaluate alternative fungicides during 2004 to ensure effective control can be maintained throughout the growing season.

Financial Benefits

The financial benefits from this work are well evident especially now that the primary cause of RMD in red beet has finally been determined. However, it remains too early to judge the full economic impact of the work as effective control measures for d. mildew still need to be sought. The primary infection period for the disease is still not known and financial losses could continue to occur in crops during 2004 and beyond. There continues therefore to be a strong economic justification for continued work to:-

- identify the most effective fungicides against d. mildew, taking due account of the possible risk and occurrence of fungicide resistance in pathogen populations

- to investigate the timing of infection in beet crops in order that fungicides can be applied most effectively and economically.

In the absence of such assurance some growers are already either reducing acreage of red beet in high risk areas or retiring from crop production altogether. If the RMD problem were to reappear at high levels in subsequent seasons without adequate safeguards the disease would undoubtedly be of considerable economic concern to many red beet growers. It is strongly recommended that one or more 'blight' fungicides eg Invader or Ranman are included in the SOLA programme.

Finally, further work should be conducted now the PCR assay is available in conjunction with 'tagging' studies to elucidate the key infection periods during the season. This information can then be used to target fungicide application more effectively to keep overall crop protection costs to a minimum.

Action Points for Growers

- Be aware of the risk from RMD in red beet and the potential economic significance should it occur.
- Monitor crops closely in 2004 for the early signs of downy mildew, root malformation or other possible symptoms that may be associated with the problem.
- Look at the economics of soil sterilisation on the farm and consider this for high risk situations ie land which has grown beet intensively in the last few years.
- 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 relating to this important problem in the crop.
- Where possible, provide continued support to ongoing research and development into RMD where the aim is to further demonstrate a link between d. mildew infection and RMD and to evaluate and secure a broader range of cost-effective fungicides for season-long economic control of the problem.

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 put economic losses due to RMD at around £1M/annum.

During the period 1999-2001 HDC sponsored a 2-year investigation at Stockbridge House. 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 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.

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 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 requires further investigation.

The primary aim of the project in 2003 project was 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 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 primary aim 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.

During January-March 2004 work was instigated at CSL in an attempt to prove the hypothesis that the RMD affected roots were a result of a systemic invasion by the obligate oomycete pathogen *Peronospora farinosa*.

Materials & Methods

Following extensive discussion with industry representatives two sites for trial purposes were identified on commercial farms in South Yorkshire. At each site half the area was treated with the soil sterilant product metham sodium (Discovery) by Sands Agricultural Services Ltd (Countrywide Farmers). Red beet seed cultivars Darko (Site 1 – Westwoodside) & Crimson Globe (Site 2 – West Butterwick) were drilled in early May and a range of fungicide and related treatments applied on a replicated basis almost immediately (Table 1). All sprays were applied using purpose-designed tractor-mounted equipment. Spray treatments were applied at approximate 4 week intervals aiming to provide broad protection from drilling through to maturity. A diary of the various applications at each of the trial sites is presented in Table 2.

Table 1 : List of fungicides and related treatments applied to the two field trial sites during 2003

Product	Active Substance	Rate of application (product/ha)	Water volume (litres/ha)	No. & timing of applications
1. Untreated (water) control	-	-	500	5 at monthly intervals
2. SL567A + Amistar	Metalaxyl-M + azoxystrobin	0.22l + 1.0l#	500	5 at monthly intervals
3. SL567A	Metalaxyl-M	1.3l	500	1 post-drilling
4. SL567A	Metalaxyl-M	0.22l	500	5 at monthly intervals
5. Fubol Gold	Metalaxyl-M + mancozeb	1.9kg	500	5 at monthly intervals
6. Invader	Dimethomorph + mancozeb	2.0kg	500	5 at monthly intervals
7. Basilex	Tolclofos-methyl	5.0kg* & 3.0kg	500	5 at monthly intervals
8. Amistar	Azoxystrobin	1.0l	500	5 at monthly intervals
9. Bavistin DF	Carbendazim	1.1l	500	5 at monthly intervals
10. Biomex + Vitomex	*Trichoderma* spp. + phosphite	1.0l + 4.0l#	500	5 at monthly intervals

** 1st application only, all subsequent applications at the lower rate.*
 # tank-mix application

Table 2 : Diary of various actions undertaken at the two field trial sites during 2003

Action	Site 1 (Westwoodside)	Site 2 (West Butterwick)
Soil sterilisation treatment	April 2003	April 2003
Drilling date	6 May	7 May
Cultivar	Darko	Crimson Globe
1st fungicide application	8 May	8 May
2nd fungicide application	4 June	4 June
Plant vigour & disease assessment	23 June	-*
3rd fungicide application	7 July	7 July
Plant vigour & disease assessment	23 July	23 July
4th fungicide application	5 August	5 August
5th fungicide application	4 September	4 September
Plant vigour & disease assessment	4 September	4 September
Plant vigour & disease assessment	7 October	-
Harvest & final assessments	3 December	21 November

** crop not emerged sufficiently for assessments to be conducted*

Trial Design

At each site a 0.5 acre plot area was sterilised with metham sodium (Discovery) by Sands Agricultural Services. An adjacent 0.5 acre area was left unsterilised for comparison.

A fully replicated extensive trial was subsequently superimposed over the two sterilised and unsterilised areas at each of the two trial sites. Each trial comprised 10 treatments x 6 replicates with 3 replicates in each of the two sterilised & unsterilised plot areas with each plot comprising of 3 rows (or beds) 20 m long. Plot size = 108 m^2 (see trial plan for precise details of trial layout).

Spray application

Sprays were applied at approximate 4 weekly intervals (5 in total) in 500 litres water/hectare using purpose-designed tractor-mounted equipment. The first application was made pre-emergent immediately after drilling. Subsequent applications targetted the developing foliage.

Assessment methodology

Both trial crops were monitored regularly for the occurrence of any disease symptoms and where found detailed assessments were carried out. Initially random 'grab' samples were taken to estimate plant vigour, the incidence of downy mildew, RMD or any other pathogens though later in the year in-crop assessments were conducted in detail. At harvest a one-metre bed width was lifted, assessed for disease incidence and crop yields recorded. Throughout the trial period the trial crops were monitored for the appearance of phytotoxicity symptoms and where present recorded and assessed.
Details of the assessment scales used are presented below:-

Plant Vigour Indices

Plant vigour was assessed using different scales depending on crop growth stage. Initially a 0-5 scale was used but later assessments relied on a 0-3 vigour scale:-

0-5 scale

0 = plant dead
1 = severe red leaf, plants at cotyledon stage or 1st true leaf
2 = Leaves small 3-4 true leaf stage, no root development and 3-5mm diameter
3 = Leaves slightly larger, ca. 5 true leaves and root development commencing, 7-8mm diameter
4 = Plants larger and more vigorous with ca. 6 true leaves root development occurring ca. 1cm diameter
5 = Excellent plant vigour ca. 7-8 true leaves, plants starting to bulk up, roots 1-2cm diameter.

N.B. This vigour assessment largely relevant to sites with infection by *Aphanomyces cochlioides*

0-3 scale

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

RMD Index

See Appendix 3 for detailed assessment scale for RMD severity

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

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

In addition, where specific symptoms were observed in crops (as indicated by growers themselves) individual plants with characteristic symptoms e.g. brown petioles, crown infection with d. mildew, leaf distortion etc were tagged using labels adjacent to the roots so that we could re-locate them in the field at a later date. This enabled monitoring of specific symptoms over time relative to the development of root distortion or RMD symptoms.

Statistical Analysis

Data from the replicated trials was input into ARM 7 management software (Gylling Data Management) and analysed statistically. The results of these analyses are presented in the tables of results.

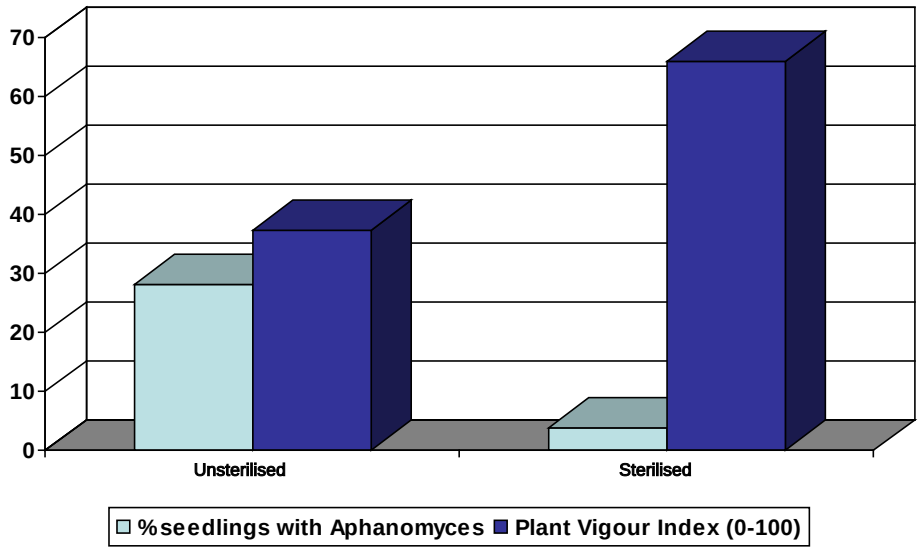
Results

(i) Progress of replicated trial sites with fungicide treatments

Trial Site 1 (Westwoodside)

At this site seedling establishment was relatively poor, especially in some low-lying 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 all the affected seedlings and was considered to be the primary cause for the establishment problems at this site. Perhaps not surprisingly there was a marked difference in this regard between the sterilised and unsterilised plots (Figure 1 & Tables 1A-1B in Appendix I). Unfortunately, none of the individual applied chemical treatments in the trial provided effective control of the disease and, whilst some may have given a slight reduction in disease severity, no significant differences were recorded (P=0.05).

Figure 1 : Severity of *Aphanomyces* infection at trial Site 1 (Westwoodside), its impact on plant vigour and efficacy of sterilisation, June 2003

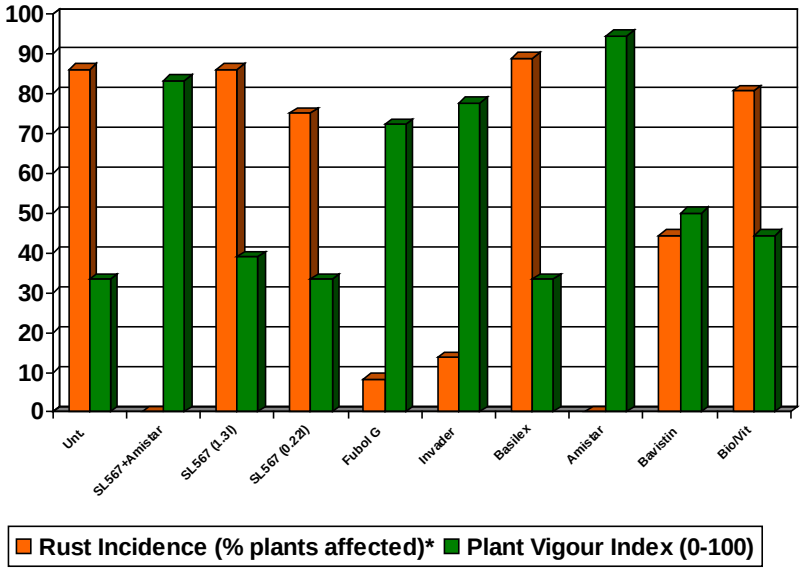


Because of the high drilling density at this site sufficient plants survived to justify taking the trial through to crop maturity. Whilst low levels of downy mildew developed on the foliage/crown tissues of occasional plants in the trial area by July relatively few RMD symptoms could be found (Tables 2A-2B, 3A-3B & 4A in Appendix 1) and where present the symptoms were very mild and this could have been caused by other factors. However, it was interesting to note that there appeared to be a relatively strong correlation between plants with visible infection by d. mildew and the occurrence of distortion on the roots at this stage. By crop maturity (from early October onwards) there was only a low level of RMD in any of the trial plots and no clear significant difference between any of the treatments (Table 7 – Appendix 1). Apart from a general effect in overall plant vigour (due largely to the impact of the *Aphanomyces*) the only other visible effects in the crop were a marked reduction in weed growth in the sterilised area compared with the non-sterilised area (especially noticeable during the crop establishment phase) and a differential incidence and severity of the leaf disease rust (*Uromyces betae*), with some of the applied treatments providing effective control. By early December some plots appeared to remain more vigorous with strong top growth compared to other less vigorous plots where the foliage had died back following early frosts (Plate 5).

Plate 5 : Prolonged greening and improved plot vigour in treated plots, November 2003

Interestingly, an assessment of plot vigour made on 3 December (Table 7 – Appendix 1) indicated a strong correlation between the applied treatments with Amistar, Fubol Gold, Invader and, to a lesser extent, Bavistin providing significantly improved crop vigour. Close inspection of the disease assessment data also shows a strong correlation with rust (*Uromyces betae*) in the trial crop (Figure 2) and this almost certainly accounts for the improved foliage vigour late in the crop. In the case of Fubol Gold and Invader it is considered that the dithiocarbamate component of the fungicide mixture is likely to have provided considerable protectant activity against this disease.

Figure 2 : Relationship between the incidence of the leaf disease rust (*Uromyces betae*) and overall plant vigour at Site 1 (Westwoodside) in late Autumn 2003



** sterilised plots only included in this assessment*

Due to the lack of significant development of RMD in this trial site during the season, the absence of downy mildew or any other potential cause for root malformation and indications from an initial lift of

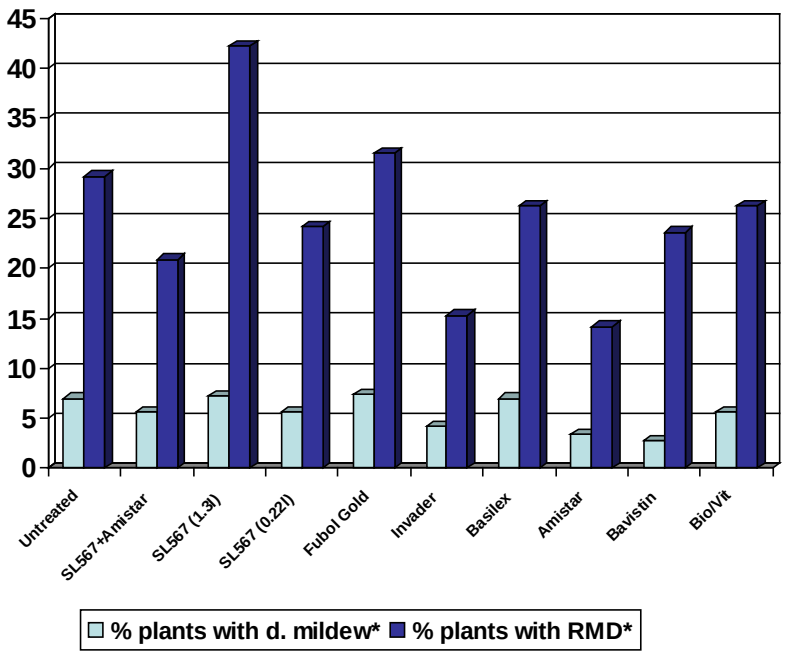
the untreated plots in late Autumn a final harvest assessment for root distortion was not conducted at this site. Throughout this trial site no symptoms of phytotoxicity were reported for any of the applied treatments.

Trial Site 2 (West Butterwick)

At this site seedling germination was slower than at Site 1 and this was due in part to the relatively wet heavy land used for the site and emergence occurred over a longer time period. Ultimately, plant density at this site was much lower though it did not suffer any apparent problems with *Aphanomyces cochlioides*. Downy mildew was found on occasional plants in the trial site on 23 July in both an in-crop assessment and an assessment of a random 'grab' sample of 10-12 plants/plot (Table 5-6A & 5-6B – Appendix 1). No significant differences were evident between treatments at this stage of the trial though, interestingly, the detailed assessment *in situ* at this early stage in the trial hinted at a possible correlation between the presence of this obligate pathogen and the occurrence of early RMD symptoms on the same 'infected' plants.

D. mildew continued to occur at low-moderate levels as the season progressed though surprisingly, the infection appeared, during routine visual inspection and assessment of sub-samples from individual plots, to occur irrespective of any of the applied fungicides and significant differences between treatments were not noted at the different assessment dates (Figure 3 & Table 8 – Appendix 1).

Figure 3 : Incidence of downy mildew and RMD in trial site 2 (West Butterwick) September 2003



** Mean of unsterilised and sterilised plots*

A final 'harvest' assessment was conducted at this site on 24-25 November when plant vigour, % plants with downy mildew, the incidence and severity of RMD symptoms and crop yield were recorded in 1metre long bed lengths in each plot (Figures 4-5 & Tables 9-10A&B – Appendix 1). In terms of crop vigour there was considerable variability and differences between treatments were non-significant. The various fungicide applications proved largely ineffective at this site though d. mildew levels were not particularly high and RMD levels remained low-moderate at maturity. Again, no significant differences were noted between the various treatments. It is noted that, in the case of metalaxyl, resistance has been reported in several oomycete pathogens eg potato blight, downy

mildew in lettuce and this could potentially account for the lack of significant differences between the results with this active substance.
In the unsterilised area Invader appeared very effective against both d. mildew & RMD (Figure 4). However, a similar result was not achieved in the sterilised trial area (Figure 5) and this variability makes interpretation of the trial data very difficult. Generally, the incidence of d. mildew and RMD was reduced in the sterilised area, as compared to the equivalent unsterilised plots, though was not eliminated completely (Figure 6). This suggests that there may be a soil-borne phase to the disorder though, at the same time, also indicates that there may be other inoculum sources which has allowed the problem to occur even in sterilised plots.

Figure 4 : Incidence of downy mildew and RMD – mean of <u>unsterilised</u> plots at Site 2 (West Butterwick) November 2003

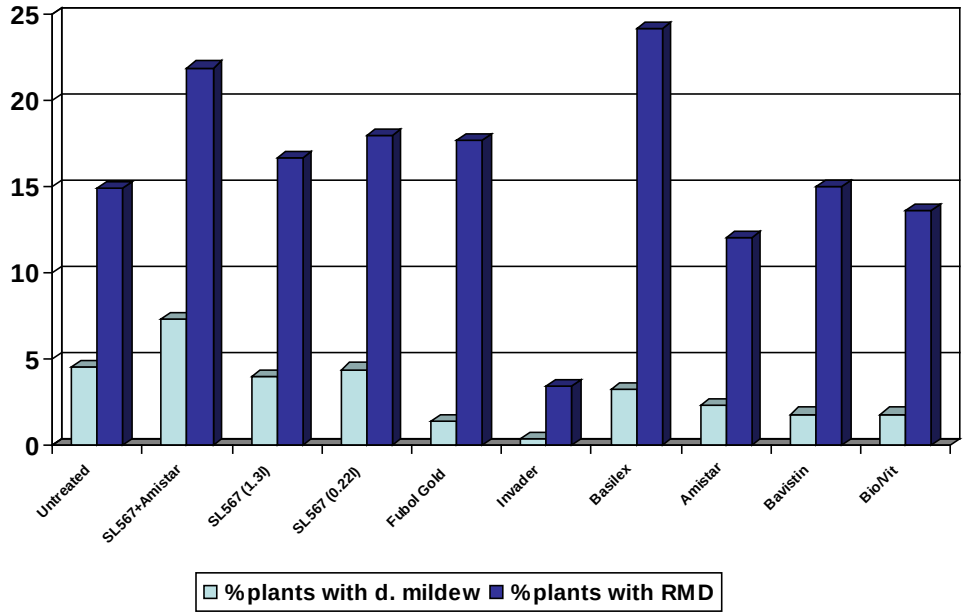


Figure 5 : Incidence of downy mildew and RMD – mean of <u>sterilised</u> plots at Site 2 (West Butterwick) November 2003

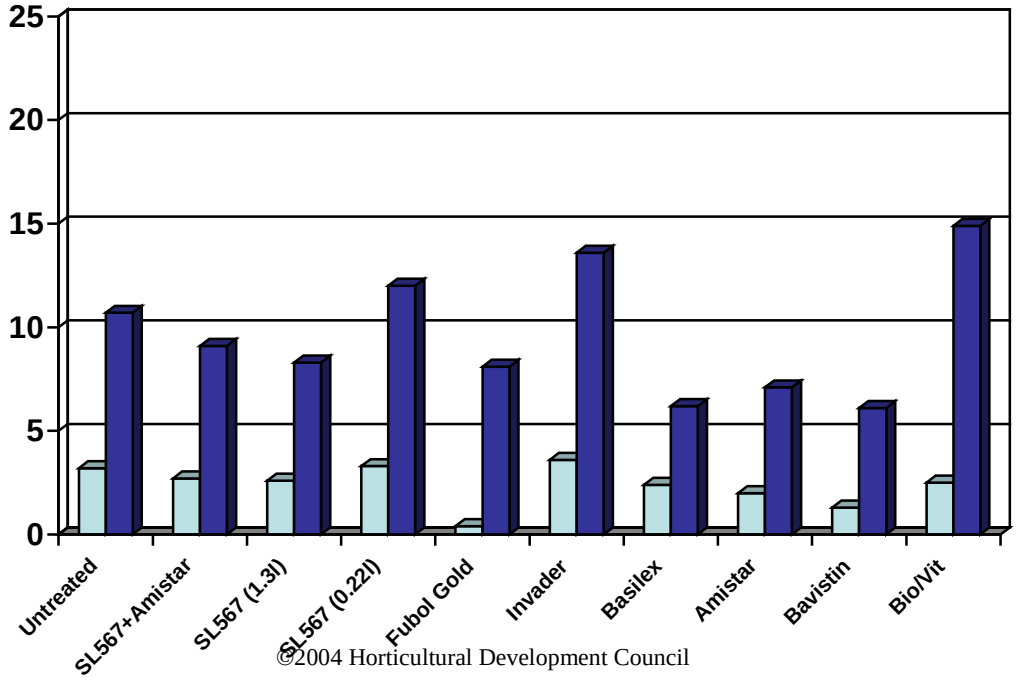
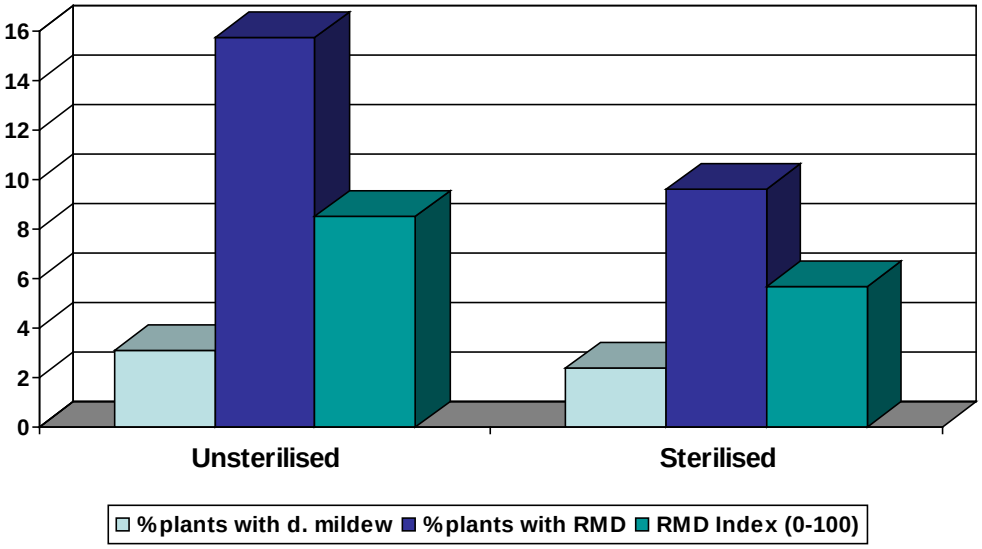
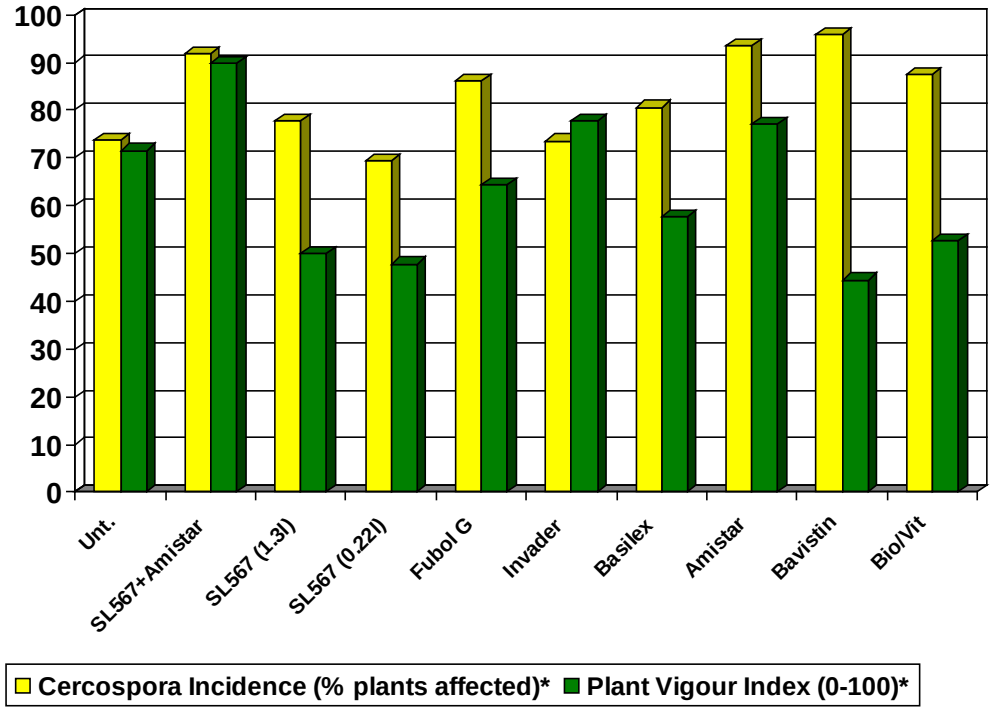


Figure 6 : Incidence of downy mildew and RMD – Mean of unsterilised and sterilised treatments at Site 2 (West Butterwick), November 2003



An improved plant vigour was also noted in specific plots at this site in late Autumn though this appeared to be unrelated to the presence of leaf disease in the crop. Unlike at Site 1, there was little rust at this site and instead *Cercospora* leaf-spot predominated. This appeared not to be well controlled with any of the applied fungicides (Figure 7) and there appeared to be little or no correlation between this disease and plant vigour in late November 2003. The improved plant vigour observed in certain plots at site 2 cannot therefore be accounted for at this stage.

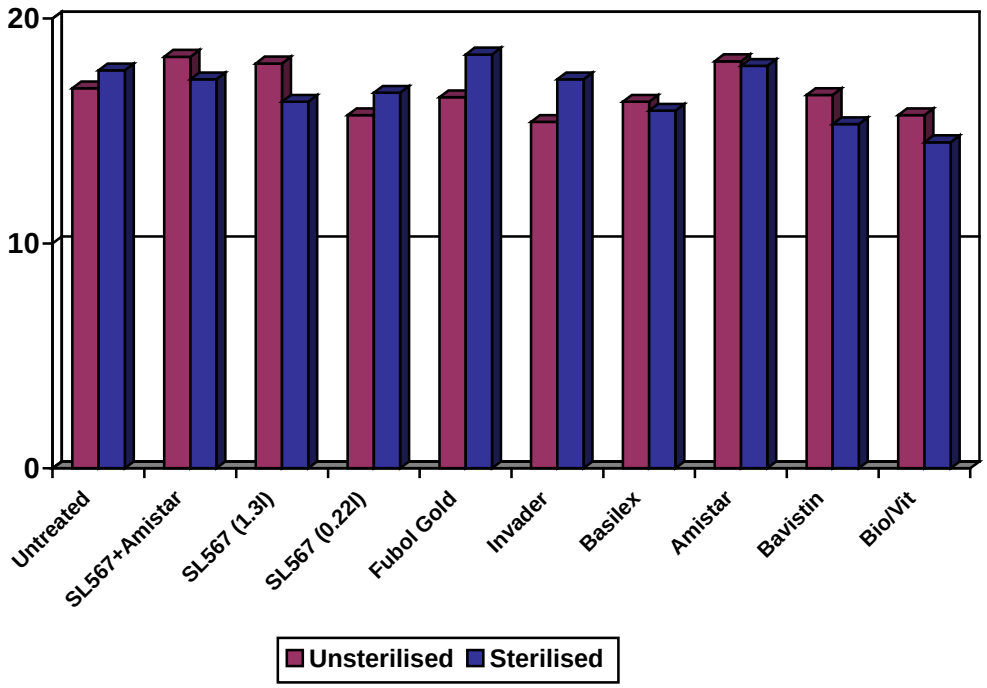
Figure 7 : Incidence of *Cercospora* leaf-spot and plant vigour at trial site 2 (West Butterwick), Autumn 2003



** Mean of unsterilised and sterilised plots*

Finally, yield data collected at this site indicated that there had been little impact of the various fungicide treatments on the total bulk weight of the crop (Figure 8). Even the soil sterilisation treatment appeared to have little effect in this regard. Again, throughout the trial period no symptoms of phytotoxicity were reported for any of the applied treatments.

Figure 8 : Total yield (kg/plot*) for unsterilised and sterilised plots at Site 2 (West Butterwick) November 2003 [For comparisons between treatments only]



** Yield not comparable with those achieved commercially as data based on limited harvest from individual trial plots. The high yield reported also due to high proportion of beet in larger size grades at this trial site.*

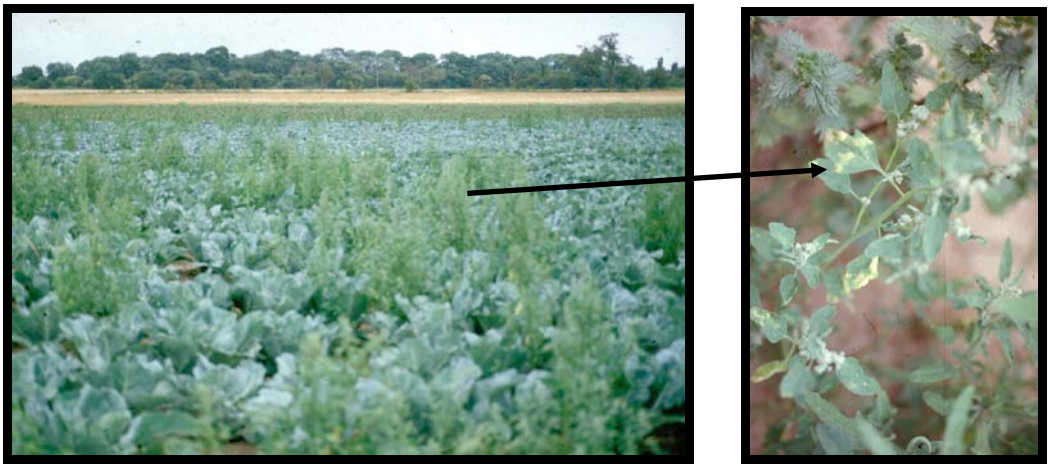
In summary, at this trial site downy mildew was the predominant pathogen to occur at appreciable levels, though it was not particularly well controlled by the various applied fungicides, and this is particularly surprising and disappointing. It may be that the 4-week interval between applications was insufficient and a shorter time between sprays may be required in future.

RMD did occur in this trial crop though was somewhat sporadic and variable in its occurrence. In the early stages of the trial there appeared to be a good correlation between d. mildew infected plants and the development of RMD though this effect appeared to become less clear as the season progressed. By the end of the trial it was evident that RMD symptoms had been suppressed to a limited extent following soil sterilisation and this implies a possible soil-borne phase to the problem. Application of the various fungicides gave mixed results which cannot be readily explained, though this may relate to the relatively poor control of d. mildew.

(ii) <u>The role of weed hosts for downy mildew development</u>

Downy mildew has also been observed at high levels in recent years on *Chenopodium album* or 'fat-hen' a common weed in UK agriculture, including in red beet crops (Plate 6a & 6b). Whether this strain was the same as that infecting red beet remained uncertain at commencement of the work programme though it was considered that the increased acreage of 'set-aside' in the last 5 years could potentially have accounted for an upsurge in both the weed host and inoculum of this air-borne pathogen.

Plate 6 : Occurrence of downy mildew in the common weed 'fat-hen' (*Chenopodium album*) adjacent to a field of red beet in the Isle of Axholme, 2003.



A detailed search of the scientific literature using an extensive series of key-words (downy mildew, *Peronospora farinosa*, *Peronospora schactii*, *Chenopodiaceae*, *Chenopodium album*, beet, beetroot, red beet, *Beta vulgaris*, sugar beet, goosefoot, fat-hen, cross-inoculation, spinach, *Spinacia oleracea*, chard, weeds, strain variation) has now demonstrated that they are in fact different strains of *P. farinosa* as follows:-

- *Peronospora farinosa* f. sp. *betae* on Red Beet & Sugar Beet
- *Peronospora farinosa* f. sp. *chenopodii* on Quinoa and other *Chenopodium* species, including *C. album* or 'fat-hen'
- *Peronospora farinosa* f. sp. *spinaciae* on Spinach (*Spinacia oleracea*)

The inference from this historic cross-inoculation work (references provided) is that the downy mildew (*P. farinosa* f.sp. *chenopodii*) observed on 'fat-hen' or goosefoot (*Chenopodium album*) in beet crops presents no risk to red beet or sugar beet crops in the vicinity as these are only susceptible to the host specialised form of the pathogen (*P. farinosa* f.sp. *betae*).

(iii) Other observations in Red Beet crops during 2003

During Autumn 2002 and during the 2003 cropping season it became evident during close examination of affected crops and in talking with industry representatives that other symptoms were, on occasions, associated with RMD. RMD affected plants frequently, though not exclusively, developed an orange-brown discoloration of the petioles (Plate 7). The symptom was considered very similar to the colour generated on beet leaves known to be systemically infected in the crown. It should be noted however that, in some cases, this brown petiole symptom may also be a varietal characteristic and therefore cannot be used alone to distinguish RMD affected plants in a crop.

Plate 7: Orange-brown discoloration of petioles in RMD affected & downy mildew infected red beet. The brown petiole symptom is often observed alone in plants with no visible mildew symptoms.

In some crops, particularly during the dry summer conditions of 2003, it was possible to identify RMD affected roots by the erect nature of the central crown leaves. Whereas healthy plants were wilting due to a significant soil moisture deficit the central leaves of RMD affected plants remained erect. This may be due to the reduced sugar content[1] of the leaves or possibly a response from the thicker tap root present on many of the RMD affected plants.

(i) Tagging plants

In one commercial crop inspected during late August 2003 the incidence of downy mildew was found to be relatively high. It was decided to 'tag' plants which showed 3 distinct symptoms:-

4. Healthy plants, no downy mildew or other symptoms visible
5. Crown infection with downy mildew, sporulation clearly evident
6. Brown petiole symptom, no sporulation of downy mildew

The plants in the 3 categories above were identified and tagged on 4 September and then the crop was left undisturbed until near harvest at which time the plants in the three categories were lifted and returned to the laboratory for detailed assessment. At the time the plants were lifted on 7 October there was a relatively high incidence of RMD. Many of the plants were wilting due to a high soil moisture deficit yet RMD affected plants were more prominent because they stood more erect in the crop.

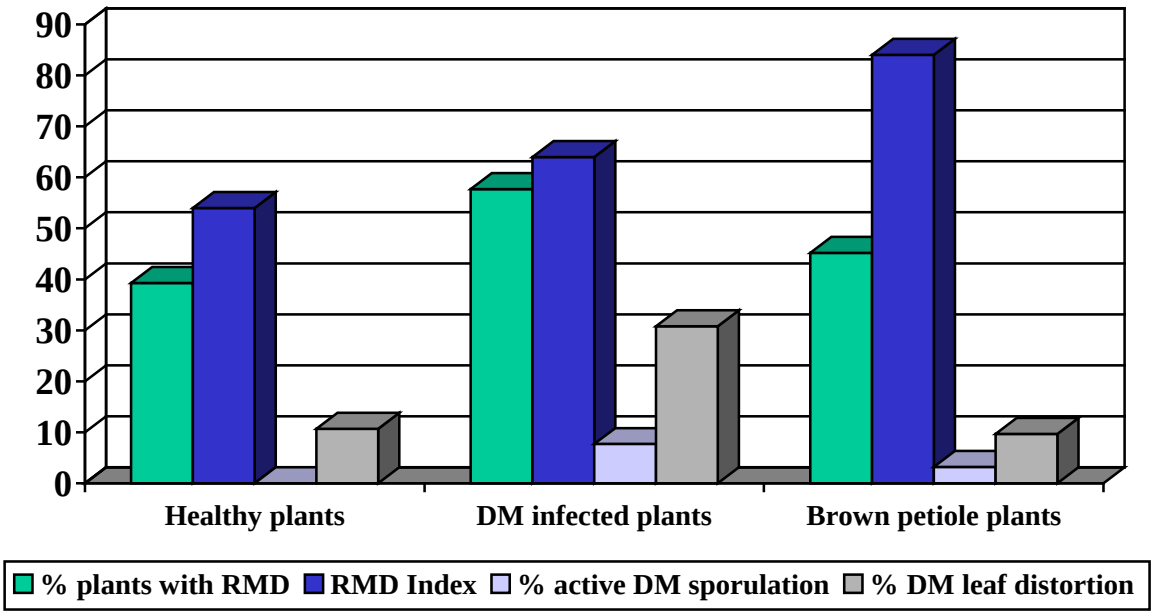
Results of the assessments are presented in Table 3 and Figure 9.

[1] *Separately, the grower co-ordinator submitted samples of RMD affected and healthy roots for independent analysis of sugar levels. A considerable (ca. 30%) reduction in sugar levels in the distorted roots, relative to those unaffected, was found.*

Table 3 : Harvest assessments from tagged plants in a commercial crop exhibiting a range of different symptoms possibly associated with RMD

Harvest Assessments	Healthy	Downy mildew infected	Brown petiole affected
% of tagged plants which developed RMD	39.3	57.7	45.2
RMD Index (distortion severity) on tagged plants	2.7	3.3	4.2
% tagged plants with active sporulation of d. mildew	0.0	7.7	3.2
% tagged plants with leaf distortion due to d. mildew	10.7	30.8	9.7
% of tagged plants with d. mildew sporulation & RMD	0.0	7.7	3.2
% of tagged plants with d. mildew leaf distortion and RMD	7.1	30.8	9.7

Figure 9: Representation of observational studies in a commercial red beet crop during 2003



Interestingly, on the plants which were visibly free of d. mildew infection at the time of tagging 39% developed RMD symptoms (Table 3 & Figure 9). This could either be due to latent infection not visible at the time of tagging or that they became infected later after the tagging had been completed. There was a much higher incidence of RMD in plants which were visibly infected with d. mildew at the time of tagging and this is highly significant. Also, those plants with a brown petiole symptom also had a higher incidence of RMD, though interestingly in this study, the disease index (RMD severity) was also greatly increased.

It may be appropriate to undertake more of this type of 'tagged' monitoring in subsequent crops to further investigate a possible link between the various symptoms.

(ii) Monitoring the 'brown petiole' symptom

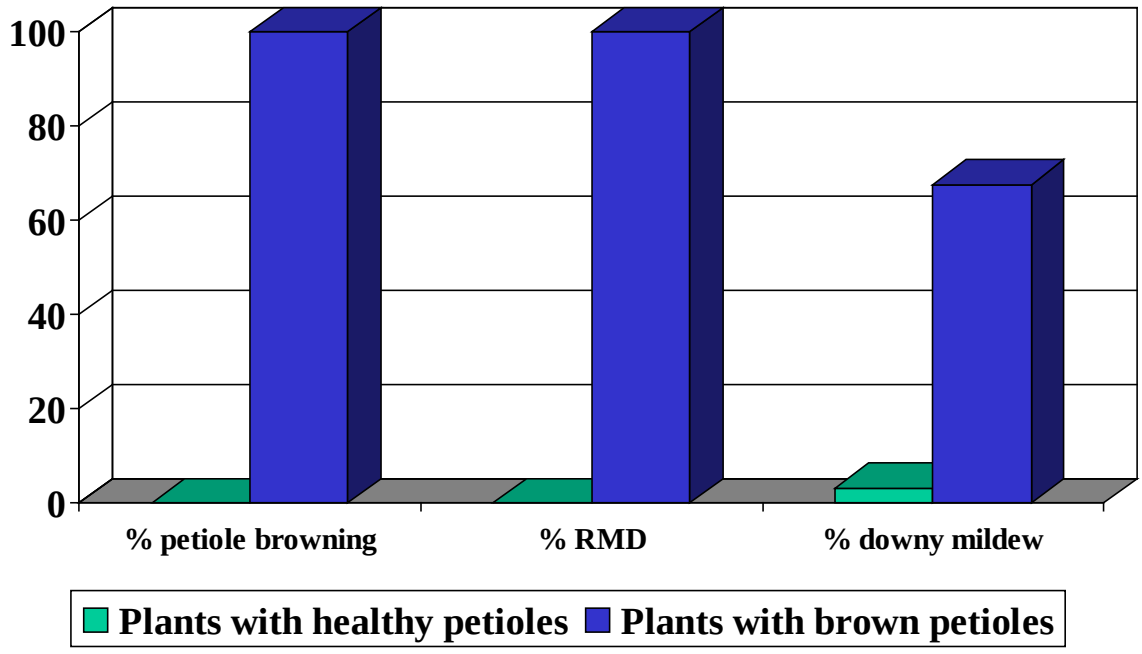
In a separate observational study in a red beet crop which exhibited brown petiole symptoms in November 2003 we collected 10-15 healthy and 10-15 'brown petiole' plants from 2 random areas of the crop. We returned these to the laboratory for assessment of RMD and associated symptoms., the results for which are presented in Table 4 & Figure 10.

What is particularly interesting is that there again appeared to be a strong correlation between the incidence of the 'brown petiole' symptom and RMD. Where healthy plants were randomly selected from this crop ie no 'brown petiole' evident, the incidence of RMD was zero. However, in contrast, in all cases where plants were selected which exhibited a 'brown petiole' symptom RMD was present on the root. Of even more significance was that we found a very low incidence (3.3%) of downy mildew on the plants with healthy petioles whereas on the plants with 'brown petiole' symptoms 68% of the plants exhibited d. mildew in the crown tissues.

Table 4 : Assessment of plants with or without brown petiole symptoms selected from a commercial field crop.

Sample No.	% petiole browning	% RMD	% downy mildew
Healthy petiole 1	0.0	0.0	0.0
Healthy petiole 2	0.0	0.0	6.7
MEAN	**0.0**	**0.0**	**3.3**
Petiole browning 1	100.0	100.0	63.6
Petiole browning 2	100.0	100.0	71.4
MEAN	**100.0**	**100.0**	**67.5**

Figure 10 : Observations of a possible relationship between the brown petiole symptom and RMD in a commercial crop in the Isle of Axholme in 2003.



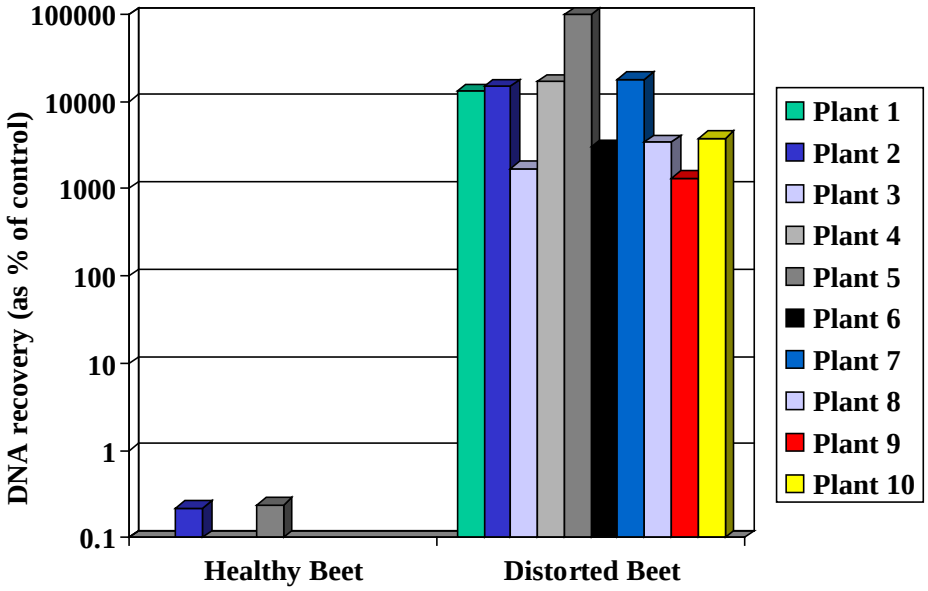
Preliminary results for the various trials and observations during 2003 were mixed though there was considerable evidence for a possible link between systemic infection with d. mildew and RMD. It was

particularly unfortunate (from an experimental basis) that 2003 proved to be unusual climatically and not conducive to the development of wet weather diseases such as d. mildew as this marred our chances of securing valuable data on fungicide performance against RMD in red beet.

There is no doubt that the potential association between d. mildew and RMD needs resolving for the industry with the utmost of urgency as this would allow other in-depth investigations to be undertaken to find effective control measures. It was therefore regarded as critical that every effort was made to determine whether RMD affected roots were systemically infected with *Peronospora farinosa* f. sp. *betae.* Following preliminary discussions with scientists at CSL a recommendation was made for HDC to separately sponsor a small investigation during winter 2003-4 to evaluate a potential novel approach to pathogen detection using DNA based technology ie PCR (Polymerase Chain Reaction). Providing initial investigative & development work was successful the novel technique could potentially be harnessed and used specifically to confirm or refute the current hypothesis that *P. farinosa* is the primary incitant of RMD. This would allow subsequent fungicide work in 2004 to be much more focused on controlling a specific primary target.
During January-March 2004, it proved possible for CSL scientists to develop an assay for *Peronospora farinosa*. This new molecular, PCR based, technique works by detecting and amplifying a unique piece of the pathogens DNA profile. In this way it can be used to confirm the presence of a specific pathogen without the need to culture onto artificial growing media. After validation against this and other pathogens, it was used to check healthy and affected red beet for systemic infection with *P. farinosa*. In the initial test 10 healthy and 10 affected beet were taken randomly from a stored crop and processed using the PCR-Taqman assay (Plate 8).

Plate 8 : Selection of healthy (left) and RMD affected (right) roots taken randomly from a red beet store in January 2004 for PCR-Taqman assay against *P. farinosa*.

Figure 11 : Results of PCR-Taqman assay for the initial 10 healthy and RMD affected roots taken from a red beet store in January 2004.



The results from the PCR-Taqman study at CSL proved to be extremely enlightening (Figure 11). An initial test taking tissue from the crown region in each root showed 70% of the RMD affected roots to be systemically infected with *P. farinosa* (data not presented). A subsequent study on individual beet demonstrated a higher recovery of DNA of *P. farinosa* in the lower section of the root. When the test was repeated on the same two lots of 10 roots 100% proved to be infected systemically with *P. farinosa*. None of the non-distorted roots yielded DNA of *P. farinosa*. Whilst it will be necessary to conduct further tests on affected & healthy roots during the 2004 this initial test, especially in conjunction with in-field observations, provides the strongest evidence yet that RMD is caused by a systemic invasion by the d. mildew fungus *P. farinosa*. In light of this new information it becomes even more imperative to evaluate alternative fungicides during 2004 to ensure effective control can be maintained throughout the growing season.

Discussion

One of the most difficult challenges in terms of elucidating the primary cause of RMD in red beet has been the sporadic nature of the problem. Since 1998, the problem has been particularly severe in only 2 years (1998 & 2002) and this has created a specific challenge in terms of conducting replicated trials to elucidate the primary cause and identify effective control measures using fungicide applications. The work conducted in 2003 provides a good example of this, as generally, disease levels in the beet crop were relatively low. At the Westwoodside site no RMD occurred in the trial crop itself, though the unsterilised plots were severely damaged by *Aphanomyces* infection. At the Butterwick site we managed to avoid any *Aphanomyces* problems though here d. mildew occurred sporadically and the climatic conditions during the season were not particularly favourable to its development. Whilst some RMD did appear in the crop it was scattered throughout the trial site and no clear differences became evident following fungicide application. Ironically, perhaps, *ad hoc* monitoring of other 'non-trial' sites during 2003 provided more valuable information regarding a possible link between d. mildew and root distortion than the replicated field trials themselves.

Whilst we were unable to secure strong evidence for fungicide efficacy against d. mildew (or RMD) from the various fungicide treatments we were able to demonstrate a significant indirect benefit of fungicide treatment in terms of late season plant vigour, especially at Site 1 (Westwoodside) and it is interesting to note that even in early Spring the following year the Amistar treated plots were clearly evident, providing much improved vigour with less crown-rotting compared to other treatments.

Because of the observed potential correlation between d. mildew incidence on plants and subsequent development of root distortion symptoms (and because of the problems associated with isolating obligate (non-culturable) pathogens such as d. mildew from such distorted root tissue) a different approach was adopted in early Spring 2004. Following discussions with scientists at CSL it became evident that one possibility was to develop a novel molecular (or PCR) technique for *Peronospora farinosa* whereby we could try and measure the amount of the unique DNA of this pathogen in healthy and distorted red beet roots. With additional financial support from HDC, CSL set about developing a novel molecular test and, within a relatively short-time period, succeeded. Following validation using known P. farinosa infected material a small no. of healthy and RMD-affected roots were provided to CSL for PCR testing and the industry awaited the results with considerable anticipation. The initial results couldn't have been clearer as all the distorted (RMD) roots had a high level of DNA of *P. farinosa* within the root tissues (signifying a deep-seated systemic infection) whereas the visibly healthy (non-distorted) root had negigible recovery levels. This result provides the strongest evidence yet for a link between early d. mildew infection and subsequent root distortion in red beet.

Contact with the Sugar Beet industry via Brooms Barn also suggests that root distortion may also be occurring in this crop following a series of 'tagging' experiments in crops (Dr M Asher, pers comm). Here, plants with crown infection with d. mildew appear to develop longitudinal fissures in the roots rather than the gross distortion that occurs in red beet. Perhaps of more significance is the fact that initial analytical studies by red beet growers in conjunction with the processors suggest that the sugar level of distorted roots is reduced significantly. If further evidence can be gathered to support this it might provide further impetus for the Sugar Beet industry to seek effective control measures for d. mildew in the

crop and this would have a significant indirect impact on fungicide availability for red beet growers.

Unfortunately, however, because of the serious nature of the disease in red beet the industry cannot afford to wait for the sugar beet industry to act and therefore must continue to seek effective control of d. mildew using novel fungicides. Now that the evidence for a strong link between d. mildew and RMD has been demonstrated, albeit in a limited number of samples, it is imperative that work is conducted to demonstratre fungicide efficacy and to secure On- or Off-Label approval for novel oomycete fungicides. Fortunately, ther has been significant developments in fungicide availability for the control of blight (*Phytophthora infestans*) in potato and, because this pathogen is an oomycete, it could reasonably be expected that the same products would be effective against d. mildew pathogens such as *Peronospora farinosa*. Because of the relatively high reported incidence of fungicide resistance in oomycete pathogens (including both the potato blight pathogen and d. mildews) it will be important to provide robust control measures incorporating fungicide mixtures containing contact (multi-site) and systemic (usually single-site) active substances (e.g. Fubol Gold : metalaxyl-M & mancozeb) rather than straight products containing a single active substance (e. g. metalaxyl-M) and to provide alternative fungicides with different modes of action (e.g. dimethomorph, cyazofamid, fluazinam etc) The emphasis on future work therefore must be as follows:_

- comparison of fungicides to demonstrate efficacy against d. mildew and RMD symptoms
- evaluation of different timings of fungicide application to determine the optimum frequency for effective control
- support On- or Off-Label approvals aiming to secure effective control measures incorporating good anti-resistance strategies.
- continuation of crop monitoring or ‘tagging’ to confirm the association between d. mildew infection and root distortion symptoms
- molecular analysis of healthy and affected plant tissues to confirm the link between d. mildew infection and RMD, including associated symptoms e.g multiple crowning, brown petioles etc.

It is therefore recommended that work on RMD is extended for another season (2004) in conjunction with scientists at CSL to further elucidate the cause of RMD and to identify and develop cost-effective control measures for the UK industry.

Conclusions

- In 2003 RMD levels were relatively low commercially compared to previous bad years (1998 & 2002).
- The trial sites chosen developed either neglible or low-moderate levels of the root distortion symptoms during the season. Site 2 (Westwoodside had high but variable levels of disease).
- *Aphanomyces cochlioides*, cause of blackleg, developed at one site though the applied fungicides were ineffective in preventing infection and much of the trial site was lost.
- Some of the applied fungicides at this site, especially Amistar, controlled rust infection effectively and this prolonged leaf area late in the season and plant survival over-winter was greatly improved.
- At the second site where both d. mildew and RMD occurred, albeit at a relatively low level, none of the applied fungicides appeared to significantly reduce infection levels with either d. mildew or RMD on the roots. However, as in the other site, plot differences were evident and over-winter plant survival was increased in some plots though here there did not appear to be a direct correlation with specific treatments.
- Crop monitoring at other commercial sites provided a strong correlation between the incidence of d. mildew infection and subsequent development of symptoms considered to be associated with the disorder of RMD.
- Evidence from a novel molecular PCR test developed at CSL provided the strongest evidence yet that RMD is caused by a systemic invasion of the root tisues by d. mildew as high levels of DNA of the fungus were found in the internal root tissues of distorted roots wheras negligible levels were found in the internal tissues of healthy roots. Further data to support these initial findings will be required.
- In the event that d. mildew is the primary incitant of RMD then it is considered that the timing of fungicide application may be critical and a 4-week interval (as applied in 2003) may be too long to maintain effective protection. A shorter 10-14 day interval may be more appropriate in subsequent studies.
- In terms of fungicide availability some progress has been made alongside this project and SOLAs for Wakil (seed treatment), SL567A and Amistar will assist the industry to some extent in minimising disease & RMD risk. However, both Wakil and SL567A rely largely on the activity of metalaxyl-M for control of d. mildew though, at this stage, we do not know whether the pathogen has developed resistance to this fungicide. Amistar whilst having a different mode of action is largely protectant and not particularly effective against oomycetes. It is considered important that the industry has access to 'blight' fungicides such as Fubol Gold, Invader & Ranman as these are more likely to provide season-long control and prevent or minimise the resistance risk due o their different modes of action and the presence of the dithiocarbamate mancozeb in the formulated products.

Technology Transfer

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. In addition to this, an A4 leaflet outlining the initial results and positive outcome of the initial molecular testing was prepared by the Project Leader and disseminated by HDC to all levy payers with an interest in red beet.

In addition, various articles have been published in HDC News and the trade press to update the industry of progress.

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.

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.

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

These trials could not have been conducted on the scale achieved without the excellent assistance from Graham & Russell Smith who not only 'engineered' and provided the commercial spray rig but also provided one of the trial sites and input a significant amount of their valuable time to assist with trial layout and spraying. Thanks also to David & Chris Moore who provided the site for the second trial undertaken in 2003; the enthusiasm from the various growers involved in the work has been much appreciated over the season. Soil sterilisation, seed and fungicide treatments were generously supplied 'in kind' by Sands Agricultural Services (now Countrywide Farmers), Elsoms seeds and the crop protection industry respectively.

Appendices

Appendix 1 : Raw data tables

Appendix 2 : Trial plan

Appendix 3 : Assessment scale for RMD symptoms

Appendix 1

Table 1A : In crop plant vigour and disease assessment at Site 1 (Westwoodside) - 23 June 2003

Treatment	Soil disinfection (+/-)	Plant Vigour Index	% seedlings with *Aphanomyces*	Incidence of d. mildew (-/+)
1. Untreated	-	40.0[a]	31.7[a]	-
2. SL567A+Amistar	-	46.7[a]	11.7[a]	-
3. SL567A (1.3l)	-	33.3[a]	47.7[a]	-
4. SL567A (0.22l)	-	40.0[a]	21.7[a]	-
5. Fubol Gold	-	33.3[a]	18.3[a]	-
6. Invader	-	33.3[a]	31.7[a]	-
7. Basilex	-	40.0[a]	38.3[a]	-
8. Amistar	-	26.7[a]	37.7[a]	-
9. Bavistin DF	-	40.0[a]	21.7[a]	-
10. Biomex/Vitomex	-	46.7[a]	21.7[a]	+
Mean		**38.0**	**28.2**	
LSD (P=0.05)		20.14	29.78	-
SD		11.74	17.36	-
CV(%)		30.89	61.55	-
1. Untreated	+	66.7[a]	3.7[a]	++
2. SL567A+Amistar	+	73.3[a]	1.7[a]	-
3. SL567A (1.3l)	+	60.0[a]	8.3[a]	-
4. SL567A (0.22l)	+	73.3[a]	1.7[a]	-
5. Fubol Gold	+	53.3[a]	2.3[a]	-
6. Invader	+	60.0[a]	8.3[a]	+
7. Basilex	+	66.7[a]	0.3[a]	-
8. Amistar	+	60.0[a]	7.0[a]	-
9. Bavistin DF	+	73.3[a]	1.7[a]	++
10. Biomex/Vitomex	+	73.3[a]	3.3[a]	-
Mean	**+**	**66.0**	**3.8**	
LSD (P=0.05)		16.31	8.57	
SD		9.51	4.99	
CV(%)		14.4	130.2	

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

Table 1B : Mean Data for in crop plant vigour and disease assessments at Site 1 (Westwoodside) - 23 June 2003 [Mean of unsterilised and sterilised treatments in Table 1A]

Treatment	Plant Vigour Index	% seedlings with *Aphanomyces*	Incidence of d. mildew
1. Untreated	53.0[a]	17.7[a]	++
2. SL567A+Amistar	60.0[a]	6.7[a]	-
3. SL567A (1.3l)	50.0[a]	28.0[a]	-
4. SL567A (0.22l)	57.0[a]	11.7[a]	-
5. Fubol Gold	44.0[a]	10.3[a]	-
6. Invader	47.0[a]	20.0[a]	+
7. Basilex	47.0[a]	19.3[a]	-
8. Amistar	43.0[a]	22.4[a]	-
9. Bavistin DF	57.0[a]	11.7[a]	++
10. Biomex/Vitomex	60.0[a]	12.5[a]	+
Mean	**51.8**	**16.0**	
LSD (P=0.05)	11.47	14.65	
SD	9.83	12.56	
CV(%)	18.90	78.39	

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

Table 2A : Laboratory assessment of plant vigour based on random 'grab' sample from each plot in central replicate of Site 1 (Westwoodside) – 23 June 2003

Treatment	Soil disinfection (+/-)	Mean no. of true leaves/plant	Mean plant height (cm)	Mean root diameter (mm)
1. Untreated	-	5.8	15.2	4.4
2. SL567A+Amistar	-	6.2	21.9	6.9
3. SL567A (1.3l)	-	6.3	16.7	3.9
4. SL567A (0.22l)	-	5.1	13.1	3.5
5. Fubol Gold	-	7.2	24.0	7.2
6. Invader	-	6.5	18.5	5.1
7. Basilex	-	6.0	21.2	6.2
8. Amistar	-	6.2	15.2	3.9
9. Bavistin DF	-	5.4	15.1	4.0
10. Biomex/Vitomex	-	6.3	18.9	5.8
Mean	**-**	**6.1**	**18.0**	**5.6**
1. Untreated	+	7.2	31.1	6.7
2. SL567A+Amistar	+	7.5	32.9	7.5
3. SL567A (1.3l)	+	7.0	31.2	8.2
4. SL567A (0.22l)	+	7.7	33.3	7.7
5. Fubol Gold	+	7.1	25.4	7.0
6. Invader	+	6.5	32.0	7.0
7. Basilex	+	7.1	32.5	7.4
8. Amistar	+	7.3	27.9	6.4
9. Bavistin DF	+	7.7	30.1	8.3
10. Biomex/Vitomex	+	7.0	29.5	6.4
Mean	**+**	**7.2**	**30.6**	**7.3**

NB : Statistical analysis of the data not possible as assessment based on data from central replicate only at this stage.

Table 2B : Mean data for laboratory assessment of plant vigour based on random 'grab' sample from each plot in central replicate of Site 1 (Westwoodside) – 23 June 2003 [Mean of unsterilised and sterilised treatments in Table 2A]

Treatment	Mean no. of true leaves/plant	Mean plant height (cm)	Mean root diameter (mm)
1. Untreated	6.5	23.1	5.6
2. SL567A+Amistar	6.9	27.4	7.6
3. SL567A (1.3l)	6.7	24.0	6.1
4. SL567A (0.22l)	6.4	23.2	5.6
5. Fubol Gold	7.2	26.0	7.1
6. Invader	6.5	25.3	6.1
7. Basilex	6.6	26.9	6.8
8. Amistar	6.8	21.6	5.2
9. Bavistin DF	6.6	22.6	6.2
10. Biomex/Vitomex	6.7	24.2	5.9
Mean	**6.66**	**24.28**	**6.20**

NB : Statistical analysis of the data not possible as assessment based on data from central replicate only at this stage.

Table 3A : In crop plant vigour and disease assessment at Site 1 (Westwoodside) - 23 July 2003

Treatment	Soil disinfection (+/-)	Plant Vigour Index (0-100)	No. of seedlings with downy mildew	% seedlings infected with d. mildew showing early symptoms of RMD *
1. Untreated	-	44.4 [a]	0.0 [a]	0.0
2. SL567A+Amistar	-	55.5 [a]	0.0 [a]	0.0
3. SL567A (1.3l)	-	44.4 [a]	0.0 [a]	0.0
4. SL567A (0.22l)	-	44.4 [a]	0.0 [a]	0.0
5. Fubol Gold	-	55.5 [a]	0.0 [a]	0.0
6. Invader	-	55.5 [a]	0.0 [a]	0.0
7. Basilex	-	44.4 [a]	0.0 [a]	0.0
8. Amistar	-	44.4 [a]	0.0 [a]	0.0
9. Bavistin DF	-	33.3 [a]	0.0 [a]	0.0
10. Biomex/Vitomex	-	55.5 [a]	0.0 [a]	0.0
Mean	**-**	**47.8**	**0.0**	**0.0**
LSD (P=0.05)		23.86	-	-
SD		13.91	-	-
CV(%)		29.11	-	-
1. Untreated	+	66.7 [a]	1.3 [a]	50.0
2. SL567A+Amistar	+	66.7 [a]	0.3 [a]	33.3
3. SL567A (1.3l)	+	66.7 [a]	0.3 [a]	33.3
4. SL567A (0.22l)	+	66.7 [a]	1.3 [a]	16.7
5. Fubol Gold	+	66.7 [a]	0.0 [a]	0.0
6. Invader	+	66.7 [a]	0.0 [a]	0.0
7. Basilex	+	66.7 [a]	0.0 [a]	0.0
8. Amistar	+	66.7 [a]	0.0 [a]	0.0
9. Bavistin DF	+	66.7 [a]	2.3 [a]	38.9
10. Biomex/Vitomex	+	66.7 [a]	2.7 [a]	44.4
Mean	**+**	**66.7**	**0.8**	**21.7**
LSD (P=0.05)		-	1.77	-
SD		-	1.03	-
CV(%)		-	123.7	-

Means followed by the same letter in the suffix do not differ significantly (P = 0.05) (Student-Newman-Keuls)
** There was a relatively strong correlation between the plants with downy mildew infection of the crown tissues (sterilised plots only) and the presence of initial RMD-like symptoms. However, levels were too low for a valid statistical comparison.*

Table 3B : Mean data for in crop plant vigour and disease assessments at Site 1 (Westwoodside) - 23 July 2003 [Mean of unsterilised and sterilised treatments in Table 3A]

Treatment	Plant Vigour Index	No. of seedlings with downy mildew	% seedlings infected with d. mildew showing early symptoms of RMD *
1. Untreated	55.6[a]	0.7[a]	25.0
2. SL567A+Amistar	61.1[a]	0.1[a]	16.7
3. SL567A (1.3l)	55.6[a]	0.1[a]	16.7
4. SL567A (0.22l)	55.6[a]	0.7[a]	8.4
5. Fubol Gold	61.1[a]	0.0[a]	0.0
6. Invader	61.1[a]	0.0[a]	0.0
7. Basilex	55.6[a]	0.0[a]	0.0
8. Amistar	55.6[a]	0.0[a]	0.0
9. Bavistin DF	50.0[a]	1.2[a]	19.5
10. Biomex/Vitomex	61.1[a]	1.4[a]	22.2
Mean	**57.22**	**0.42**	**10.83**
LSD (P=0.05)	11.33	1.00	-
SD	9.71	0.86	-
CV(%)	16.97	206.20	-

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

Table 4A : Laboratory assessment of plant vigour based on random 'grab' sample (10 plants/plot) from each plot in central replicate of Site 1 (Westwoodside) – 23 July 2003

Treatment	Soil disinfection (+/-)	Mean plant height (cm)	Mean root diameter (mm)	Mean Incidence of Downy Mildew (%)	Mean RMD Severity Index (0-100)*
1. Untreated	-	Assessment not conducted due to the high incidence of *Aphanomyces* in the unsterilised plots and the absence of other pathogens			
2. SL567A+Amistar	-				
3. SL567A (1.3l)	-				
4. SL567A (0.22l)	-				
5. Fubol Gold	-				
6. Invader	-				
7. Basilex	-				
8. Amistar	-				
9. Bavistin DF	-				
10. Biomex/Vitomex	-				
1. Untreated	+	45.7 [a]	25.3 [a]	0.0 [a]	1.3
2. SL567A+Amistar	+	45.1 [a]	23.1 [a]	0.0 [a]	0.0
3. SL567A (1.3l)	+	44.6 [a]	20.2 [a]	0.0 [a]	1.5
4. SL567A (0.22l)	+	45.7 [a]	25.6 [a]	0.0 [a]	1.0
5. Fubol Gold	+	45.6 [a]	20.7 [a]	0.0 [a]	1.0
6. Invader	+	45.5 [a]	21.9 [a]	3.3 [a]	2.0
7. Basilex	+	49.1 [a]	25.5 [a]	0.0 [a]	2.5
8. Amistar	+	46.2 [a]	26.0 [a]	0.0 [a]	2.5
9. Bavistin DF	+	48.2 [a]	25.0 [a]	3.3 [a]	2.0
10. Biomex/Vitomex	+	47.6 [a]	25.3 [a]	3.3 [a]	4.0
LSD (P=0.05)		8.59	10.24	5.42	-
SD		5.01	5.97	3.16	-
CV(%)		10.8	25.0	316.2	-

Means followed by the same letter in the suffix do not differ significantly (P = 0.05) (Student-Newman-Keuls)
** A negligible level of root distortion was observed at this site during progress with the trial over the season. Due to the low levels of RMD observed the data has not been analysed statistically.*

Table 5A : Plant vigour and disease assessment conducted in the crop at Site 2 (West Butterwick) - 23 July 2003

Treatment	Soil disinfection (+/-)	Plant Vigour Index (0-100)	Mean no. of seedlings with downy mildew/m row	% seedlings infected with d. mildew showing early symptoms of RMD *
1. Untreated	-	100.0 [a]	2.0 [a]	41.7
2. SL567A+Amistar	-	100.0 [a]	2.0 [a]	72.2
3. SL567A (1.3l)	-	88.8 [a]	2.7 [a]	83.3
4. SL567A (0.22l)	-	100.0 [a]	4.0 [a]	52.2
5. Fubol Gold	-	100.0 [a]	3.3 [a]	80.6
6. Invader	-	100.0 [a]	1.0 [a]	66.7
7. Basilex	-	100.0 [a]	3.7 [a]	95.2
8. Amistar	-	100.0 [a]	1.0 [a]	22.2
9. Bavistin DF	-	100.0 [a]	1.7 [a]	33.3
10. Biomex/Vitomex	-	100.0 [a]	0.7 [a]	33.3
Mean	**-**	**98.9**	**2.1**	**58.1**
LSD (P=0.05)		10.44	2.54	-
SD		6.09	1.48	-
CV(%)		6.15	67.36	-
1. Untreated	+	88.8 [a]	1.3 [a]	66.7
2. SL567A+Amistar	+	100.0 [a]	2.0 [a]	20.0
3. SL567A (1.3l)	+	77.7 [a]	1.3 [a]	66.7
4. SL567A (0.22l)	+	100.0 [a]	3.0 [a]	46.7
5. Fubol Gold	+	77.8 [a]	1.7 [a]	16.7
6. Invader	+	88.8 [a]	1.3 [a]	8.3
7. Basilex	+	88.8 [a]	1.7 [a]	20.0
8. Amistar	+	88.8 [a]	0.7 [a]	33.3
9. Bavistin DF	+	88.8 [a]	0.3 [a]	0.0
10. Biomex/Vitomex	+	77.7 [a]	1.3 [a]	0.0
Mean	**+**	**87.8**	**1.4**	**27.8**
LSD (P=0.05)		24.36	3.19	-
SD		14.20	1.86	-
CV(%)		16.18	126.8	-

Means followed by the same letter in the suffix do not differ significantly (P = 0.05) (Student-Newman-Keuls)
** This data provides an indication of potential correlation between the incidence of d. mildew in the trial area and the subsequent development of RMD symptoms. However, infection levelswith d. mildew/RMD remained too low for a valid statistical comparison.*

Table 5B : Mean data for in crop plant vigour and disease assessment at Site 2 (West Butterwick) - 23 July 2003 [Mean of unsterilised and sterilised treatments in Table 5A above]

Treatment	Plant Vigour Index	Mean No. of seedlings with downy mildew/m row	% seedlings infected with d. mildew showing early symptoms of RMD *
1. Untreated	94.4[a]	1.7[a]	50.0
2. SL567A+Amistar	100.0[a]	2.0[a]	46.0
3. SL567A (1.3l)	83.3[a]	1.5[a]	79.2
4. SL567A (0.22l)	100.0[a]	3.5[a]	51.0
5. Fubol Gold	88.9[a]	2.1[a]	48.5
6. Invader	94.4[a]	1.2[a]	37.5
7. Basilex	94.4[a]	2.7[a]	57.7
8. Amistar	94.4[a]	0.9[a]	27.7
9. Bavistin DF	94.4[a]	1.0[a]	16.7
10. Biomex/Vitomex	88.9[a]	1.0[a]	16.7
Mean	**93.33**	**1.83**	**42.96**
LSD (P=0.05)	12.35	1.84	-
SD	10.58	1.58	-
CV(%)	11.34	86.21	-

Means followed by the same letter in the suffix do not differ significantly (P = 0.05) (Student-Newman-Keuls)
** This data provides an indication of potential correlation between the incidence of d. mildew in the trial area and the subsequent development of RMD symptoms. However, infection levels remained too low for a valid statistical comparison.*
For statistical comparisons of data between treatments see Table 5A above

Table 6A : Laboratory assessment of plant vigour, d. mildew incidence & RMD severity based on random 'grab' samples from each plot at Site 2 (West Butterwick) – 23 July 2003.

Treatment	Soil Sterilisation (+/-)	Mean Plant Height (cm)	Mean Root Diameter (mm)	Mean Incidence of downy mildew (%)	RMD Severity Index (0-100)*
1. Untreated	-	50.2 [a]	37.0 [a]	10.0 [a]	12.7 [a]
2. SL567A+Amistar	-	52.9 [a]	42.1 [a]	13.3 [a]	18.7 [a]
3. SL567A (1.3l)	-	51.0 [a]	37.4 [a]	16.7[a]	10.0 [a]
4. SL567A (0.22l)	-	51.3 [a]	34.3 [a]	6.7[a]	16.0 [a]
5. Fubol Gold	-	54.3 [a]	37.0 [a]	13.3 [a]	10.4 [a]
6. Invader	-	52.6 [a]	35.7 [a]	6.7[a]	9.3 [a]
7. Basilex	-	50.5 [a]	36.3 [a]	6.7 [a]	11.3 [a]
8. Amistar	-	56.8 [a]	43.8 [a]	20.0 [a]	15.4 [a]
9. Bavistin DF	-	49.6 [a]	35.2 [a]	10.0[a]	16.7 [a]
10. Biomex/Vitomex	-	54.1 [a]	35.8 [a]	3.3[a]	12.0 [a]
Mean		**52.3**	**37.5**	**10.7**	**13.3**
LSD (P=0.05)		5.50	8.85	21.27	8.89
SD		3.21	5.16	12.40	5.18
CV(%)		6.13	13.77	115.8	39.13
1. Untreated	+	50.2 [a]	35.3 [a]	3.3 [a]	4.7 [a]
2. SL567A+Amistar	+	54.7 [a]	37.5 [a]	3.3 [a]	4.7 [a]
3. SL567A (1.3l)	+	54.7 [a]	37.6 [a]	10.0 [a]	10.7 [a]
4. SL567A (0.22l)	+	50.1 [a]	31.4 [a]	3.3 [a]	3.3 [a]
5. Fubol Gold	+	53.4 [a]	38.3 [a]	10.0 [a]	4.7 [a]
6. Invader	+	54.4 [a]	39.7 [a]	11.1 [a]	11.0 [a]
7. Basilex	+	51.9 [a]	37.7 [a]	6.7 [a]	3.3 [a]
8. Amistar	+	56.1 [a]	36.1 [a]	3.3 [a]	6.0 [a]
9. Bavistin DF	+	52.3 [a]	34.9 [a]	3.3 [a]	5.3 [a]
10. Biomex/Vitomex	+	50.0 [a]	35.4 [a]	6.7 [a]	9.3 [a]
Mean		**52.8**	**36.4**	**6.1**	**6.3**
LSD (P=0.05)		4.57	9.72	15.86	8.10
SD		2.67	5.67	9.25	4.72
CV(%)		5.05	15.56	151.36	74.85

Means followed by the same letter in the suffix do not differ significantly (P = 0.05) (Student-Newman-Keuls)
** Assessment of RMD based on early signs of root distortion on 12 plants/plot only as 'grab' sample. Data to be treated with caution.*

Table 6B : Mean data for laboratory assessment of plant vigour, d. mildew incidence & RMD severity based on random 'grab' samples from each plot at Site 2 (West Butterwick) – 23 July 2003 [Mean of unsterilised & sterilised treatments in Table 6A above].

Treatment	Mean Plant Height (cm)	Mean Root Diameter (mm)	Mean Incidence of downy mildew %)	RMD Index (0-100)*
1. Untreated	50.3[b]	36.1[a]	6.7[a]	8.7[a]
2. SL567A+Amistar	54.0[ab]	39.8[a]	8.4[a]	11.7[a]
3. SL567A (1.3l)	53.0[ab]	37.5[a]	13.4[a]	10.4[a]
4. SL567A (0.22l)	50.7[b]	32.9[a]	5.0[a]	10.0[a]
5. Fubol Gold	53.7[ab]	37.7[a]	11.8[a]	7.4[a]
6. Invader	53.5[ab]	37.7[a]	8.4[a]	10.0[a]
7. Basilex	51.4[b]	37.0[a]	6.6[a]	7.3[a]
8. Amistar	56.5[a]	40.0[a]	11.8[a]	10.7[a]
9. Bavistin DF	51.0[b]	35.1[a]	5.0[a]	11.0[a]
10. Biomex/Vitomex	52.0[ab]	36.9[a]	5.0[a]	10.7[a]
Mean	**52.6**	**37.2**	**8.4**	**9.78**
LSD (P=0.05)	3.39	5.97	12.10	6.22
SD	2.91	5.12	10.37	5.33
CV(%)	5.53	13.74	123.38	54.53

Means followed by the same letter in the suffix do not differ significantly (P = 0.05) (Student-Newman-Keuls)
** Assessment of RMD based on early signs of root distortion on 12 plants/plot only as 'grab' sample. Data to be treated with caution.*

Table 7 : Assessment of the leaf disease rust (Uromyces betae) in the crop on 4 October and overall plant vigour in a 'grab' sample assessed in the laboratory on 3 December at Westwoodside.

Treatment	Soil disinfection (+/-)	Incidence of rust (%) 4 October 2003	Index of RMD Severity (0-100) 4 October 2003	Plant Vigour* Index (0-100) 3 December 2003
1. Untreated	-	Assessment not conducted due to the high incidence of *Aphanomyces* in the unsterilised plots at this site and absence of other pathogens		33.3 [c]
2. SL567A+Amistar	-			83.3 [a]
3. SL567A (1.3l)	-			33.3 [c]
4. SL567A (0.22l)	-			33.3 [c]
5. Fubol Gold	-			55.6 [b c]
6. Invader	-			44.4 [c]
7. Basilex	-			33.3 [c]
8. Amistar	-			72.2 [a b]
9. Bavistin DF	-			55.6 [b c]
10. Biomex/Vitomex	-			33.3 [c]
LSD (P=0.05)		-	-	15.66
SD		-	-	9.13
CV(%)		-	-	19.11
1. Untreated	+	86.1 [a]	8.9 [a]	33.3 [b]
2. SL567A+Amistar	+	0.0 [b]	5.0 [a]	83.3 [a]
3. SL567A (1.3l)	+	86.1 [a]	7.2 [a]	38.9 [b]
4. SL567A (0.22l)	+	75.0 [a]	3.3 [a]	33.3 [b]
5. Fubol Gold	+	8.3 [b]	4.5 [a]	72.2 [a]
6. Invader	+	13.9 [b]	3.4 [a]	77.7 [a]
7. Basilex	+	88.9 [a]	2.2 [a]	33.3 [b]
8. Amistar	+	0.0 [b]	2.8 [a]	94.4 [a]
9. Bavistin DF	+	44.4 [a b]	10.5 [a]	50.0 [b]
10. Biomex/Vitomex	+	80.6 [a]	5.0 [a]	44.4 [b]
LSD (P=0.05)		30.92	7.47	16.59
SD		18.02	4.35	9.68
CV(%)		37.29	82.46	17.24

Means followed by the same letter in the suffix do not differ significantly (P = 0.05) (Student-Newman-Keuls)
** Marked differences in plot vigour wwere noted during a final crop visit in December. A detailed vigour assessment was conducted using a 0-3 scale, subsequently converted to form a vigour index (0-100). Nnote the significant correlation between earlier(November) foliar disease (rust) in the crop and subsequent plant vigour.*

Table 8 : Laboratory assessment of d. mildew, incidence & severity of RMD and leaf infection with *Cercospora* on random 'grab' samples from Site 2 (West Butterwick) – 4 September 2003.

Treatment	Soil Sterilis -ation (+/-)	% plants with downy mildew	% plants with RMD	RMD Severity Index (0-100)	% plants with *Cercospora* leaf-spot	% leaf area infection with *Cercospora*
1. Untreated	-	11.1 [a]	30.5 [a]	12.2 [b]	80.6 [a]	1.3 [a]
2. SL567A+Amistar	-	5.6 [a]	25.0 [a]	11.7 [b]	86.1 [a]	6.0 [a]
3. SL567A (1.3l)	-	6.1 [a]	48.2 [a]	34.4 [a]	69.5 [a]	0.9 [a]
4. SL567A (0.22l)	-	5.6 [a]	25.0 [a]	17.8 [a b]	75.0 [a]	1.9 [a]
5. Fubol Gold	-	12.0 [a]	35.2 [a]	13.7 [b]	83.3 [a]	4.7 [a]
6. Invader	-	5.6 [a]	16.7 [a]	5.5 [b]	61.1 [a]	2.6 [a]
7. Basilex	-	5.6 [a]	33.3 [a]	16.1 [a b]	77.8 [a]	1.9 [a]
8. Amistar	-	6.7 [a]	20.6 [a]	9.3 [b]	90.0 [a]	6.7 [a]
9. Bavistin DF	-	2.8 [a]	19.4 [a]	7.8 [b]	94.4 [a]	5.3 [a]
10. Biomex/Vitomex	-	8.3 [a]	33.3 [a]	16.7 [a b]	91.7 [a]	13.6 [a]
Mean		**6.9**	**28.7**	**14.5**	**81.0**	**4.5**
LSD (P=0.05)		14.24	22.66	13.56	37.84	9.75
SD		8.30	13.21	7.90	22.06	5.68
CV(%)		119.9	45.97	54.44	27.21	126.7
1. Untreated	+	2.8 [a]	27.8 [a]	7.2 [a]	66.7 [a]	2.5 [a]
2. SL567A+Amistar	+	5.6 [a]	16.7 [a]	5.6 [a]	97.2 [a]	4.5 [a]
3. SL567A (1.3l)	+	8.3 [a]	36.1 [a]	17.8 [a]	83.3 [a]	5.0 [a]
4. SL567A (0.22l)	+	5.5 [a]	30.6 [a]	8.9 [a]	63.9 [a]	1.1 [a]
5. Fubol Gold	+	2.8 [a]	27.8 [a]	7.2 [a]	88.9 [a]	7.4 [a]
6. Invader	+	2.8 [a]	13.9 [a]	3.9 [a]	86.1 [a]	4.7 [a]
7. Basilex	+	8.3 [a]	19.4 [a]	6.1 [a]	83.4 [a]	3.4 [a]
8. Amistar	+	0.0 [a]	8.3 [a]	3.9 [a]	97.2 [a]	2.9 [a]
9. Bavistin DF	+	2.8 [a]	30.6 [a]	10.6 [a]	97.2 [a]	7.6 [a]
10.Biomex/Vitomex	+	2.8 [a]	19.4 [a]	6.7 [a]	83.3 [a]	2.2 [a]
Mean		**4.2**	**23.1**	**7.8**	**84.7**	**4.1**
LSD (P=0.05)		10.58	25.68	11.62	29.28	6.04
SD		6.17	14.97	6.77	17.07	3.52
CV(%)		148.1	64.92	87.12	20.15	85.19

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

Table 9: In crop plant vigour assessment at Site 2 (West Butterwick) – 24 November 2003

Treatment	Soil disinfection (+/-)	Plant Vigour* Index (0-100)	Soil disinfection (+/-)	Plant Vigour* Index (0-100)
1. Untreated	-	66.7 [a]	+	77.8 [a]
2. SL567A+Amistar	-	88.9 [a]	+	88.9 [a]
3. SL567A (1.3l)	-	66.7 [a]	+	33.3 [a]
4. SL567A (0.22l)	-	38.9 [a]	+	55.6 [a]
5. Fubol Gold	-	72.2 [a]	+	55.6 [a]
6. Invader	-	88.9 [a]	+	66.7 [a]
7. Basilex	-	55.6 [a]	+	61.1 [a]
8. Amistar	-	77.8 [a]	+	77.8 [a]
9. Bavistin DF	-	38.9 [a]	+	50.0 [a]
10. Biomex/Vitomex	-	61.1 [a]	+	44.4 [a]
Mean		**65.6**		**61.1**
LSD (P=0.05)		42.34		45.90
SD		24.68		26.76
CV(%)		37.65		43.79

Means followed by the same letter in the suffix do not differ significantly (P = 0.05) (Student-Newman-Keuls)
** Marked differences in plot vigour noted during final crop visit. A detailed assessment was conducted using a 0-3 severity scale, subsequently converted to form a vigour index (0-100). See also the foliar assessment in Table 9 and note the apparent lack of correlation between plot vigour and earlier foliar disease in the crop.*

Table 10A : Final harvest assessment for d. mildew, incidence & severity of RMD and crop yield at Site 2 (West Butterwick) – 25 November 2003

Treatment	Soil Sterilisation (+/-)	% plants with downy mildew	% plants with RMD	RMD Severity Index (0-100)	Crop yield (tonnes/ha)
1. Untreated	-	4.5 [a]	14.9 [a]	9.2 [a]	93.7 [a]
2. SL567A+Amistar	-	7.2 [a]	21.67 [a]	11.5 [a]	101.8 [a]
3. SL567A (1.3l)	-	4.0 [a]	16.7 [a]	10.3 [a]	100.0 [a]
4. SL567A (0.22l)	-	4.4 [a]	18.0 [a]	12.1 [a]	87.0 [a]
5. Fubol Gold	-	1.4 [a]	17.8 [a]	9.1 [a]	91.5 [a]
6. Invader	-	0.4 [a]	3.4 [a]	1.7 [a]	85.7 [a]
7. Basilex	-	3.2 [a]	24.2 [a]	14.0 [a]	90.4 [a]
8. Amistar	-	2.3 [a]	12.0 [a]	6.8 [a]	100.4 [a]
9. Bavistin DF	-	1.8 [a]	15.0 [a]	6.4 [a]	92.2 [a]
10. Biomex/Vitomex	-	1.8 [a]	13.6 [a]	7.4 [a]	87.0 [a]
Mean		**3.1**	**15.7**	**8.8**	**93.0**
LSD (P=0.05)		6.67	18.78	11.50	26.78
SD		3.89	10.95	6.70	15.61
CV(%)		125.5	69.68	75.86	16.79
1. Untreated	+	3.2 [a]	11.1 [a]	7.3 [a]	98.5 [a]
2. SL567A+Amistar	+	2.7 [a]	9.1 [a]	5.4 [a]	96.3 [a]
3. SL567A (1.3l)	+	2.6 [a]	8.4 [a]	5.5 [a]	90.4 [a]
4. SL567A (0.22l)	+	3.3 [a]	12.3 [a]	7.6 [a]	92.6 [a]
5. Fubol Gold	+	0.4 [a]	8.1 [a]	4.9 [a]	102.2 [a]
6. Invader	+	3.6 [a]	13.8 [a]	8.7 [a]	96.3 [a]
7. Basilex	+	2.4 [a]	6.2 [a]	3.2 [a]	88.5 [a]
8. Amistar	+	2.0 [a]	7.1 [a]	3.0 [a]	99.3 [a]
9. Bavistin DF	+	1.3 [a]	6.1 [a]	3.6 [a]	85.2 [a]
10.Biomex/Vitomex	+	2.5 [a]	15.0 [a]	8.6 [a]	80.4 [a]
Mean		**2.4**	**9.7**	**5.8**	**92.9**
LSD (P=0.05)		4.44	14.80	9.60	25.79
SD		2.59	8.63	5.60	15.04
CV(%)		107.4	88.7	96.94	16.17

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

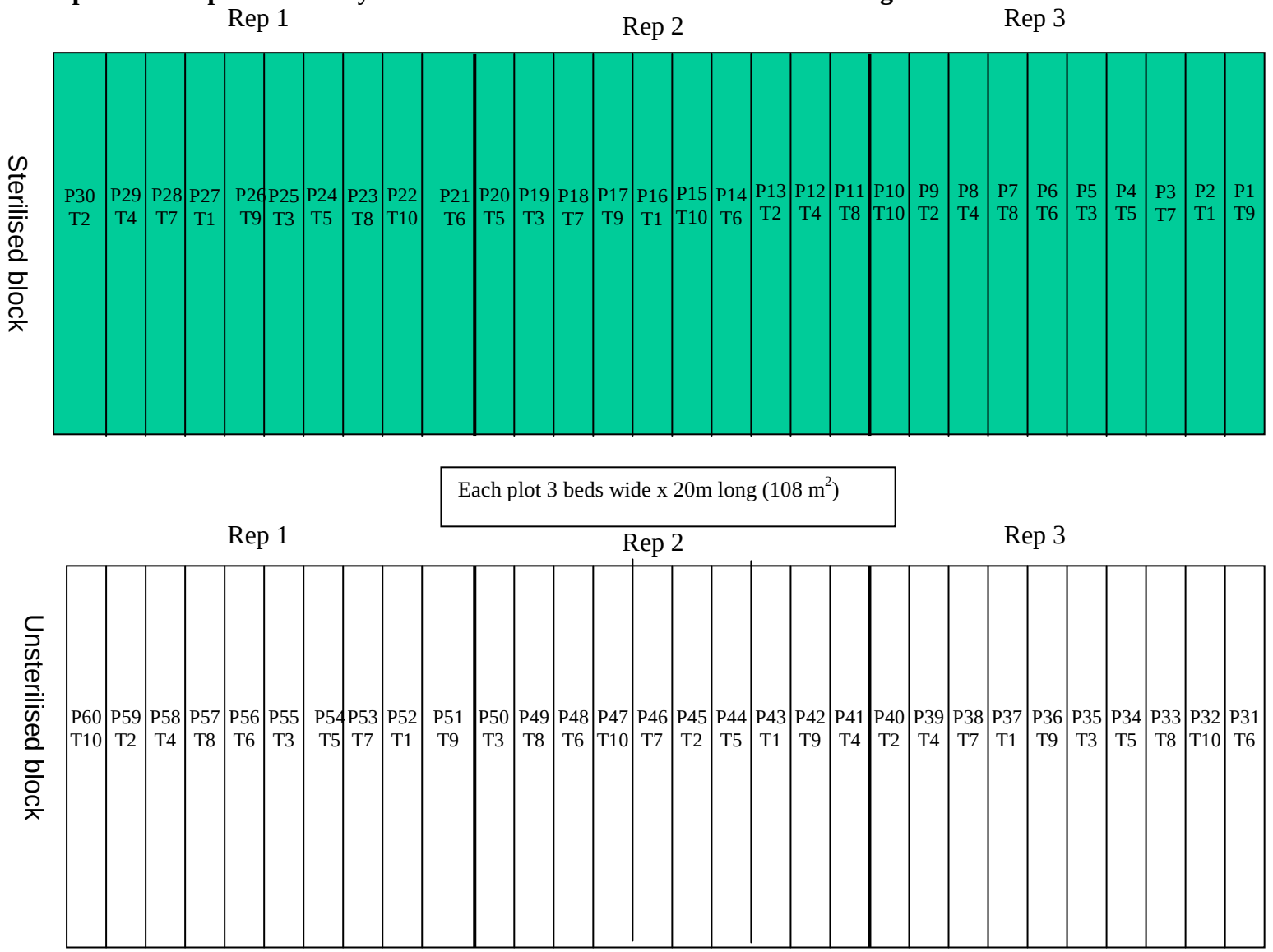
Table 10B : Final harvest assessment for d. mildew, incidence & severity of RMD and crop yield at Site 2 (West Butterwick) – 25 November 2003 [Mean of unsterilised & sterilised treatments (6 replicates/treatment).

Treatment	% plants with downy mildew	% plants with RMD	RMD Severity Index (0-100)	Crop yield (tonnes/ha)
1. Untreated	3.9[a]	12.97[a]	8.2[a]	96.1
2. SL567A+Amistar	5.0[a]	15.37[a]	8.4[a]	99.1
3. SL567A (1.3l)	3.3[a]	12.5[a]	7.9[a]	95.2
4. SL567A (0.22l)	3.9[a]	15.2[a]	9.9[a]	89.8
5. Fubol Gold	0.9[a]	12.9[a]	7.0[a]	96.9
6. Invader	2.0[a]	8.6[a]	5.2[a]	91.0
7. Basilex	2.8[a]	15.2[a]	8.6[a]	89.4
8. Amistar	2.2[a]	9.5[a]	4.9[a]	99.8
9. Bavistin DF	1.6[a]	10.6[a]	5.0[a]	88.7
10.Biomex/Vitomex	2.2[a]	14.3[a]	8.0[a]	83.7
Mean	**2.8**	**12.7**	**7.3**	**93.0**
LSD (P=0.05)	3.66	11.43	7.10	16.69
SD	3.14	9.79	6.09	14.31
CV(%)	113.83	77.01	83.33	15.39

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

Appendix 2

Example of the experimental layout for the field-scale trials undertaken during 2003.



Appendix 3 : Assessment scale for RMD symptoms