FINAL
PROJECT REPORT

FV 300

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Red Beet: Studies to confirm a link between infection by downy mildew (*Peronospora farinosa f.sp. betae*) and root malformation disorder (RMD)

April 2007

Project Title:	Red Beet: Studies to confirm a link between infection by downy mildew (*Peronospora farinosa f.sp. betae*) and root malformation disorder (RMD)
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The results and conclusions in this report are based on an investigation conducted over one year. 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.

AUTHENTICATION

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

Signature……..……

Ms C Lambourne
Project Manager
Stockbridge Technology Centre

Date……

Signature…….

Dr N Boonham
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Date ……..

Report authorised by……..

Dr G M McPherson MBPR (Hort.)
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****STOP PRESS****

STC won the 'Grower of the Year' awards in the category of 'Science into Practice' at a prestigious event in London on 22nd February 2007 organised by Haymarket publications and sponsored by Bakkavor for this work

"**Resolving the Enigma of RMD in Red Beet"**

It is important that all parties who participated in this investigation take some credit for this success. Particular mention must go to Dr Neil Boonham and his team at CSL who developed the molecular assay for *Pfb* and who undertook various PCR analyses

Also, special thanks to Graham & Russ Smith of South Carr Farm for their dedication, enthusiasm and commitment to the cause of RMD over the last few years and also to all the other growers and the various company representatives who assisted in this protracted investigation which finally allowed us to get to the bottom of the RMD problem.

Grower Summary

FV 300

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FV 300 : GROWER SUMMARY

Red Beet : Red Beet: Studies to confirm a link between infection by downy mildew (*Peronospora farinosa f.sp. betae*) and root malformation disorder (RMD)

Headlines

- Field and laboratory studies in 2006 have finally confirmed that RMD in red beet is caused by a systemic infection by the downy mildew fungus (*Peronospora farinosa* f.sp. *betae*).
- To minimise the risk from RMD growers should ensure effective protection from downy mildew using approved oomycete fungicides e.g. metalaxyl-M (SL567A), azoxystrobin (Amistar) and including seed treatment e.g. Wakil XL.
- It would be advisable to maintain season-long protection, though there was an indication from the 2006 studies that suggests that the older the plants before infection with *Pfb* the less susceptible they are to RMD symptoms.
- Growers should recognise that fungicide application once the early stages of RMD have been initiated are unlikely to prevent further damage.
- Further R&D would be necessary to fully elucidate the biology and epidemiology of the infection process, including the potential for seed-borne infection.

Background and Expected Deliverables

A series of investigations were conducted during the period 1998-2006 to investigate a number of possible causes for the severe root malformation disorder (RMD) that has had such a significant economic impact for red beet growers, especially those in the Isle of Axholme area of North Lincolnshire. Various potential causes including Rhizomania, chemical injury, nematodes, *Rhizoctonia*, *Aphanomyces, Pythium* root rot have all been eliminated as possible causes over the years. As a result of detailed observation and epidemiological study a hypothesis was raised that RMD was caused by a systemic infection by *Peronospora farinosa* f.sp. *betae*. Unfortunately, as downy mildew is an obligate pathogen (that is, it cannot be cultured on artificial agar media in the laboratory) it has proved particularly challenging to confirm this hypothesis scientifically.

Since we first speculated a potential involvement by downy mildew a range of oomycete fungicides have been secured, primarily via the HDC-funded SOLA programme. This has potentially provided growers with a short-term solution to the problem until such time that confirmation as to the cause of RMD could be made. Since a range of oomycete fungicides were approved and made available to the red beet industry it is interesting to note that neither downy mildew nor RMD have been of particular economic significance in commercial crops.

For final affirmation of cause and effect between downy mildew and RMD, confirmation via Koch's postulates is essential. Previous studies relied, to some extent, on the development of a molecular assay or TaqMan Polymerase Chain Reaction (PCR) to demonstrate the presence of DNA of *Pfb* in RMD affected beet. However, because this test does not differentiate between viable and non-viable (dead) propagules (spores, mycelium etc) it cannot be relied upon alone for confirmation of cause and effect. To confirm downy mildew as the primary cause of RMD it is necessary to introduce the pathogen into beet and reproduce characteristic symptoms...and this had not been achieved previously under experimental conditions.

This attempt at confirmatory work was undertaken at STC in 2006 rather than on grower sites as in previous years as this allowed us to introduce the downy mildew pathogen in a complex series of artificial inoculation studies in the field. The experimental design allowed as much information as possible to be gathered, not only about a potential link between inoculation with *Pfb* and symptom development, but also about infection timing e.g. seedling developmental stage and time in the season, the impact of fungicides and other cultural effects such as sowing density and soil disturbance.

Separately, we also collected a series of commercial seed samples and these were submitted for molecular analysis to further determine the risk of seed-borne infection.

Summary of the Project (2006) and Main Conclusions

A series of experimental areas in a field-grown red beet crop were established at STC during May-June 2006. Relatively large areas (ca. 500m^2) of red beet cv. Crimson Globe[1] were sown successively at weekly intervals (4 in total) from mid-late May and mid-June. Each drilled area comprised 4 x 1.8m wide beds each ca. 70m in length and 11 treatments x 4 replicate plots (44 plots/experimental area or176 plots in total) were superimposed in each drilling. The individual treatments included *Pfb* inoculations at various timings, the application of fungicides to specifically target d. mildew and various cultural amendments as proposed by industry representatives. A full list of the treatments used is available in the science section of the report.

For the first inoculation of the experimental plots inoculum of *Pfb* was 'harvested' from infected beet leaves from a naturally infected commercial crop in the Isle of Axholme. For all subsequent inoculations it was possible to harvest infected leaf material, and hence sporangia (spores), from the guard areas of the earlier inoculated experimental plots.

[1] Red Beet seed cv. Crimson Globe was kindly supplied free of charge by Elsoms seeds; their support is gratefully acknowledged.

Inoculated plots were sprayed with a spore suspension of *Pfb* and covered with polythene sheeting for 24hrs to maintain leaf wetness and enhance the chance of infection.

Both leaf and crown infections of downy mildew developed successfully in the crops following artificial inoculation, particularly at the earliest drilling date (Plate 1).

Plate 1. Early crown infection with d. mildew on young red beet seedlings.

Seedling samples were collected during the growing season from emergence through to harvest for testing using the TaqMan PCR assay, developed by CSL, to quantify the amount of *Pfb* DNA present in individual seedlings. As the seedlings developed the collected roots were assessed for the presence and severity of distortion using a 0-5 scale.

At harvest, high numbers of distorted beet were found in the *Pfb* inoculated areas (>50% of the harvested roots in some plots). Fewer, less severely affected, roots were also found in uninoculated plots and those which had been treated with oomycete fungicides. The highest incidence and severity of RMD was observed in the earliest sown crop, closely followed by those seen in the 3rd sowing (Plate 2). A relatively low incidence and severity of RMD symptoms were recorded in the 2nd and 4th sowings. However, at all four sowing dates, the highest level of RMD was observed in plots which had been inoculated with *Pfb* when the plants were between the cotyledon and 4 true leaf stage of development[2]. Seedlings which were inoculated later, at the 8-12 true leaf stage, consistently showed lower levels of RMD at all four sowing dates. These results demonstrate that seedling age and climatic conditions (linked to sowing times) play a vital role in the infection process and subsequently on RMD development in crops. This data provides growers with important information to hopefully develop useful cultural control strategies. Effective control of d. mildew on young seedlings, at least in 2006, would appear to

[2] An exception to this was recorded in the first sowing when plants were not inoculated at the cotyledon stage due to the late development of inoculum in commercial field crops.

be very important in minimising the risk from RMD development and crop rejection. Where an oomycete fungicide programme was used the incidence and severity of RMD was reduced significantly in the experimental plots and this provides confidence in the industry to continue application of approved fungicides.

The use of Wakil XL (cymoxanil + fludioxinil + metalaxyl-M) treated seed, to counter potential seed-borne infection by downy mildew and early seedling infection via air- or soil-borne sporangia, is advisable in this regard. As the level of protection afforded by such a seed treatment is limited further applications of foliar applied oomycete fungicides will be required to maintain season-long protection from RMD. Oomycete fungicides that are likely to provide a moderate-good level of protection from foliar downy mildew, crown infection and RMD are mancozeb (Dithane), metalaxyl-M (SL567A) and azoxystrobin (Amistar). Rather than relying on the use of a single active substance growers are urged to alternate with fungicides from different mode of action groups to minimise the risk of the downy mildew pathogen becoming insensitive or resistant to the applied fungicides.

To a large extent, most growers are already putting much of this information into practice based on previously speculative advice via the Red Beet Technology Group and this may have contributed to the very low incidence (approx 1% of harvested beet) of RMD affected beet which have been observed in commercial crops in the last 2-3 seasons.

Plate 2. Distorted red beet harvested from the 1st sowing at STC

Additional studies to investigate the potential risk of seed-borne d. mildew were also undertaken following the detection of *Pfb* DNA in preliminary PCR analyses on 6 samples of seed tested in 2005. Further PCR analyses were carried out on 11 additional samples in 2006 with similar levels of detection

found as in 2005. However, it is important to note that the PCR assay cannot distinguish between DNA from viable or non-viable fungal inoculum. For confirmation of these results it is necessary therefore to conduct growing-on tests under controlled conditions to demonstrate seedling infection with *Pfb* and hence pathogen viability. Unfortunately, the growing-on tests conducted in 2006 were inconclusive and no downy mildew infection was expressed on the seedlings under test. Other research groups have also reported difficulty in securing symptom expression with downy mildew from seed-borne inoculum in seedling tests (Dr L du Toit, Washington State University, *pers. comm*). Further investigation is required to finally validate these Taqman PCR results on beet seed.

Financial Benefits

As previously stated, commercial red beet growers are already putting effective strategies into practice to control RMD in their crops and this is proving successful as RMD has not been a significant economic problem in the last 2-3 seasons. The financial benefit to individual growers is therefore very large and potentially offers savings of around £1M/annum to the industry as a whole, providing effective control with fungicides is maintained.
It is, of course, also important to note that the findings from this research in 2006 will provide growers a far greater level of confidence to take the appropriate action for RMD control, especially in terms of fungicide application either as seed treatment or foliar applied products.

Action Points for Growers

- Now that the downy mildew pathogen has been proven to be the cause of RMD in red beet, growers are encouraged to adopt as many of the control strategies detailed in this report to ensure adequate control of downy mildew in their crops, particularly in the early stages of sowing and emergence when crops appear to be at most risk.
- Be aware of other beet crops (sugar beet, fodder beet, red beet), including ‘volunteer’ or ‘weed’ beet in the immediate vicinity as this potentially increases the risk of downy mildew and subsequent RMD.
- The use of seed treatment e.g. Wakil XL (cymoxanil, fludioxinil & metalaxyl-M) offers effective protection from potential seed, soil or air-borne inoculum during the early stages of the crop pre- and post-emergence. It should be used where possible until such time that further research can be undertaken to confirm the preliminary Taqman PCR results outlined in this report.
- Irrespective of earlier seed treatment, red beet crops should continue to be monitored from emergence onwards for the first signs of downy mildew, root malformation or other possible symptoms that may be associated with downy mildew infection. This is

especially relevant during periods of cool wet weather when infection conditions are at their optimum.

- Where downy mildew infection is found in crops growers should consider application of foliar oomycete fungicides e.g. mancozeb (protectant only), metalaxyl-M (SL567A) and azoxystrobin (Amistar) to maintain protection and prevent systemic invasion of the hypocotyl tissues by the fungus which is likely to lead to RMD symptoms later in the year. Where necessary, repeat applications of fungicides may be required to maintain protection during high risk periods.
- To reduce the risk of fungicide resistance growers should not rely on a single fungicide for downy mildew control. Instead, it is necessary to formulate an alternating strategy using products from different mode of action groups to reduce selection pressure. For further advice in this regard talk to your distributor, agronomist or crop consultant and refer to FRAG-UK (www.pesticides.gov.uk/rags.asp).
- R&D in 2006 suggests that seedlings are most at risk of subsequent RMD infection when they are infected by d. mildew early i.e. up to the 8-10 leaf stage. However, different weather patterns in different seasons could modify this risk. Therefore, depending on the weather patterns during the season, consider longer-term protection from d. mildew until such time that further information on infection timing and risk under different climatic conditions is available.
- Growers should recognise that fungicide application once the early stages of RMD have been initiated are unlikely to prevent further damage.
- Growers are advised to maintain good communication links to alert each other of early d. mildew infections in red beet crops. Early recognition and control of the disease in crops will result in overall reduction of inoculum (air-borne spores) in the vicinity; thereby reducing the RMD risk.
- Where possible, growers should retain sub-samples of different seed batches for future testing purposes to provide further information regarding potential link between seed-borne downy mildew and RMD.
- Continued close liaison between members of the Red Beet Technology Group, the STC/CSL research team and the appropriate HDC Technical Managers is to be encouraged to ensure the latest information, including fungicide availability for red beet, is widely disseminated throughout the industry.

SCIENCE SECTION

Introduction

The background to this study stretches back over a number of years when red beet growers reported the appearance of 'crinkly' or distorted beet during routine harvesting of crops back in autumn 1998. Mature crops showed a high incidence of what was quickly referred to as root malformation disorder or RMD even though the cause of the problem was not immediately identifiable. The severity of the root distortion was so serious in 1998 that many consignments of beet were rejected by the processors and growers subsequently spent a considerable amount of time on grading lines trying to manually separate out affected roots. As a result, the problem caused severe economic loss to many red beet growers particularly in the Isle of Axholme area of South Yorkshire and Lincolnshire. Interestingly, RMD was less problematic during 1999-2001 though was again severe in 2002. Various estimates put the economic losses due to RMD at around £1M/annum in years when the problem was particularly severe (1998 & 2002).

Following the initial occurrence of RMD, the red beet industry, via HDC, sponsored a series of investigations during 1999-2001 & 2003-2006 at STC to try and pin-point the cause of the symptoms. Studies initially commenced on a broad basis to conduct a literature search, distribute a questionnaire to growers, conduct a series of pot studies and to eliminate a number of possible factors that could potentially have led to such severe root distortion. During this initial investigation, tests for 'Rhizomania' and other virus diseases were conducted, as were tests for herbicide injury, nematode infestation and bacterial pathogens. All tests proved negative. Subsequently, a series of replicated field trials were undertaken to evaluate the potential of various fungicides. Whilst these provided some indication of a potential link with oomycete fungi, they were inconclusive due to the relatively low levels of RMD that occurred commercially during this period. However, on the basis of these initial findings recommendations were made to secure Off-Label approval for a series of oomycete fungicides via the HDC SOLA programme. As a result, a number of useful fungicides have subsequently gained approval for use on red beet and this has significantly improved the armoury of available fungicides on this relatively minor crop.

In 2002, when growers again reported that there was a high incidence of RMD in red beet crops, visits were made to affected crops and lengthy discussions undertaken regarding the timing and environmental conditions which had led to the 'bad RMD season'. These discussions began to suggest that that there might be a link between RMD and downy mildew *(Peronospora farinosa* f. sp. *betae or Pfb)*; a foliar pathogen that had been present in

beet crops for at least the last 30-40 years and probably longer. Studies in subsequent years focused on proving cause and effect for the hypothesis that *Pfb* was responsible for RMD using a range of techniques including fungicide efficacy and timing trials, soil sterilisation studies, investigations into alternate hosts, crop monitoring, artificial inoculation studies and the development of new methodologies to assist in working with this obligate pathogen. Collaborative studies also took place with the Central Science Laboratory (CSL) at York to develop molecular testing methods to provide confirmation of the field trial results. This joint work with CSL proved to be very successful and a valuable molecular (PCR) method for the detection and quantification of DNA of *Pfb* in red beet root tissues was developed. In an initial validation test, using two batches of red beet (10 healthy and RMD affected), a positive result for Pfb was obtained from the affected red beet and this further strengthened the hypothesis for an association between RMD and d. mildew infection. The full results of this initial development work were presented in a previous report (FV 226c).

Throughout this period, investigations into RMD have continued to be hampered by a combination of factors, not least the unusual aetiology and sporadic nature of the disorder. This aspect, in conjunction with the difficulty associated with obligate (non-culturable) pathogens such as downy mildew, made elucidation of the RMD problem particularly challenging.

Although the studies conducted in previous years have added much weight to the downy mildew hypothesis, it had not provided the proof necessary for a confirmatory diagnosis as to the cause of the RMD symptom largely because it had not proved possible to artificially inoculate beet in pot studies and successfully reproduce RMD symptoms i.e. Koch's postulates had not been completed. It was decided that work undertaken in 2006 must focus on achieving this goal. In 2006, STC undertook a large-scale replicated field trial using a crop of red beet cv. Crimson Globe established on site in an area where beet crops had not previously been grown (to avoid the potential risk of extraneous soil-borne inoculum) and largely remote from neighbouring beet (sugar, red & fodder) crops (and any subsequent air-borne inoculum of *Pfb*). Four sequential sowings of red beet were carried out at weekly intervals during late May through to mid-June. A series of 11 treatments were superimposed over each crop area in a bid to provide conclusive proof of cause and effect and to confirm the link between downy mildew infection and RMD. The main obstacle to be overcome in the work was developing a robust methodology for successful inoculation of red beet with the downy mildew pathogen, *Pfb*. As it transpired, there proved to be a greater problem; that of securing a viable and virulent inoculum source on commercial crops of beet in what turned out to be one of the hottest driest springs in living memory. Finally, as in the earlier studies,

we established further collaboration with CSL and used the Taqman PCR, as a substitute to conventional isolation procedures with facultative pathogens, as the final step in demonstrating the presence of the obligate pathogen, Peronospora farinosa f.sp. betae, to ultimately demonstrate Koch's postulates and prove that infection by downy mildew was responsible for root malformation disorder.

Materials & Methods

Location: Field J, Stockbridge Technology Centre, Cawood, Selby, N.Yorks, YO8 3TZ.

Crop: Red Beet – *Betae vulgaris* cv Crimson Globe (Thiram soaked, Lot 97622, supplied by Elsoms Seeds).

Trial Design

Four sequential sowings of red beet seed were carried out in the prepared trial area at STC during late May through to early June 2006. At each drilling date four beds, each approximately 70m long, were sown using an Oyjard drill on a 1.8metre wide bed system at a density of 6-7kg/acre, representative of standard commercial practice.

Within each drilling date 11 treatments with 4 replicate plots per treatment (44 plots/drilling in total) were marked out in each of the 4 trial areas in a fully randomised block design. The dimensions of individual plots were 1.83m x 2m. Regular irrigation of the established crops was carried out throughout the duration of the trial to encourage the development of the inoculated pathogen, *Peronospora farinosa* f.sp. *betae*.

Treatment List

1. Uninoculated control
2. Uninoculated fungicide control (Fubol Gold + Invader tank mix foliar sprays at 14 day intervals)
3. **Inoculated** with *Pfb* once at cotyledon stage
4. **Inoculated** with *Pfb* once at 2-4 true leaf stage
5. **Inoculated** with *Pfb* once at 8-12 true leaf stage
6. **Inoculated** with *Pfb* weekly from emergence to 8-12 TLs
7. **Inoculated** with *Pfb* weekly from emergence to 8-12 TLs + routine fungicide application with Fubol Gold + Invader (1-2 days post-inoculation).
8. Soil disturbance treatment at 1-2 true leaf stage (simulating rotovation).
9. Soil disturbance treatment at 1-2 true leaf stage (simulating rotovation) and **inoculated** with *Pfb* as in T7.
10. Second drilling within the row (by hand) at 1-2 TL stage^.
11. Second drilling within the row (by hand) at 1-2 TL stage^ followed by **inoculation** with *Pfb* as in T7

Treatments 8-11 proposed by growers based on previous field observations with RMD. T8-9 conducted by gently disturbing roots of plants using a hand trowel. T10-11 conducted by creating a shallow furrow for second sowing beside established rows (2 on left of seedlings, 2 on right) post-emergence.

Table 1: Crop Diary

Crop Event	1st Sowing	2nd Sowing	3rd Sowing	4th Sowing
Sowing date	25th May 2006	2nd June 2006	9th June 2006	16th June 2006
1st Inoculation (+ treatments applied)	20.6.06 (T3*,4,6,7,9 & 11)	27.6.06 (T3,6,7,9 &11)	27.6.06 (T3,6,7,9 &11)	11.7.06 (T3,6,7,9 &11)
2nd Inoculation (+ treatments applied)	27.6.06 (6,7,9 &11)	11.7.06 (T4,6,7,9 &11)	11.7.06 (T4,6,7, 9 & 11)	18.7.06 (T4,6,7,9 & 11)
3rd Inoculation (+ treatments applied)	11.7.06 (T5,6,7,9 &11)	18.7.06 (T5,6,7,9 &11)	18.7.06 (T6,7,9 &11)	26.7.06 (T6,7,9 &11)
4th Inoculation (+ treatments applied)	Plants at 9-10 TL stage no further inoculations.	Plants at 9-10 TL stage no further inoculations.	26.7.06 (T5,6,7,9 &11)	1.8.06 (T5,6,7,9 &11)
			Plants at 9-10 TL stage no further inoculations.	Plants at 9-10 TL stage no further inoculations.
Soil disturbance (T8 & T9)	21.6.06	28.6.06	28.6.06	12.7.06
Second drilling (T10 & 11)	21.6.06	28.6.06	28.6.06	12.7.06
1st Fungicide application	23.6.06	29.6.06	29.6.06	13.7.06
2nd Fungicide application	6.7.06	13.7.06	13.7.06	28.7.06
3rd Fungicide application	20.7.06	28.7.06	28.7.06	10.8.06
4th Fungicide application	3.8.06	10.8.06	10.8.06	25.8.06
5th Fungicide application	17.8.06	25.8.06	25.8.06	8.9.06
6th Fungicide application	31.8.06	8.9.06	8.9.06	21.9.06
7th Fungicide application	15.9.06	21.9.06	21.9.06	-
In-crop sampling (1)	18.7.06	25.7.06	25.7.06	9.8.06
In-crop sampling (2)	15.8.06	22.8.06	22.8.06	6.9.06
In-crop sampling (3)	12.9.06	18.9.06	18.9.06	4.10.06
Crop Harvest & Assessment	16.10.06	25.10.06	23.11.06	10.1.06

** T3 not applied at this stage due to an absence of inoculum of Pfb in commercial crops at this stage of the season.*

Inoculation Procedure

A spore suspension of *P. farinosa* was prepared by washing spores from heavily infected beet leaf material. Initially, infected beet leaves, collected from an infected commercial crop in the Isle of Axholme, were used to prepare inoculum for inoculation of the first drilling. However, the disease was absent from commercial crops until late May and it was not possible to inoculate the 1st drilled crop at the prescribed timing of cotyledon stage. The first inoculation of this crop occurred at the 2-4 true leaf stage. For all subsequent inoculation timings it proved possible to 'harvest' infected leaves from the guard areas of the trial crop to prepare inoculum. This was possible because the initial inoculation in late May was very successful (in no small part to the regular applied irrigation in what turned out to be one of the driest spring periods on record) and the pathogen established successfully and spread to adjacent uninoculated plants.

At each inoculation timing, the plot area to be inoculated (0.65 x 1.8m) was marked out using marker poles, the foliage sprayed initially with water to provide leaf wetness to aid the infection process, and then sprayed with approximately 100ml of the prepared spore suspension using a hand pressured Hozelock sprayer (Plate 3). The inoculated areas were then covered with polythene overnight to maintain leaf wetness and raise humidity (Plate 4).

Plate 3: Applying inoculum to plots

Plate 4. Covering plots with polythene during inoculation

Application of fungicides

Applications of a tank mix of Fubol Gold (1.9kg/ha) and Invader [3](2kg/ha) were made to T2 and T7 approximately 24 hrs after the first inoculation in each sowing. The fungicide application was subsequently repeated at 14 day intervals for the duration of the trial. Fungicides were applied using a battery powered knapsack sprayer with a boom attachment fitted with flat fan nozzles (FF110/0.80/3) and run at a constant 2 Bar pressure. The purpose of the tank mix was to counter the risk of metalaxyl-M (or dimethomorph) resistant isolates in the pathogen population and hence ensure treatment efficacy from an experimental standpoint. It is important to note that whilst metalaxyl-M is approved for use on red beet dimethomorph is not at the current time.

Soil disturbance and 2nd drilling

The soil disturbance treatments (8 & 9) were incorporated into the trial design following grower observations in commercial crops where beet seedlings, disturbed in the early stages of their development e.g. during rotovation of headlands or in tractor wheelings, often resulted in the development of distorted beet. The purpose of the treatment therefore was to try and recreate this phenomenon by disturbing seedlings using a hand trowel at or around the 1-2 true leaf stage and allowing them to re-establish.

Plate 5. 2nd drilling of red beet seedlings in plot

Similarly, a higher incidence of RMD had been observed commercially where they had been subjected to repeat sowing following poor establishment of crops due to *Aphanomyces* infection. In the field trials at STC we hand sowed additional rows of seed close to the existing rows at the 1-2 true leaf stage to try and create a growing environment where seedlings were stressed due to overcrowding.

Field Sampling and Tagging

[3] It is important to note that Invader (dimethomorph+mancozeb) is currently not approved for use on red beet.

Within each of the four sowings seedling samples were collected from each plot on 3 occasions during the trial period. Four beet were collected randomly e.g. from the central 2 rows, but not confined to the inoculated areas when these were present. Sampling commenced 4 weeks post inoculation and was repeated at 4 weekly intervals (3 sampling times/sowing) for the duration of the trial. On each sampling occasion the young beet were examined and individually scored using the 0-5 severity scale for RMD symptoms[4] (Appendix 1). The samples were then transported to CSL for sampling and storage prior to PCR analysis at a later date.

Regular inspections of the crops were carried out during the season to identify beet plants with obvious downy mildew crown infections (Plates 6 & 7). Plants found with infected crowns were 'tagged' using canes to allow them to be monitored for possible distortion effects at a later date.

Plate 6. Downy mildew crown infection Note distortion of leaves

Plate 7. Downy mildew crown infection

Harvesting

Approximately one month after the final application of fungicides to treatments 2 & 7 in each sowing the crop was harvested. All beet in an 80cm (the length of the inoculated area) x 1.8m area in each plot were lifted. The total number of beet harvested/plot was recorded. Any distorted beet were scored using the 0-5 severity scale (Appendix 1). Any beet which had been tagged at an earlier date in the trial e.g. had been subject to a downy mildew crown infection were also harvested and scored separately to determine any potential correlation between crown infection and RMD development.

[4] The 0-5 severity scale was converted to a 0-100 severity index using the following formula:

$$\frac{0x(0) + 1x(1) + 2x(2) + 3*(3) + 4x(4) + 5 \times (5)}{\text{Total number of samples collected}} \times \frac{100}{5}$$

Seed Testing

A total of 11 batches of seed from crops sown during the 2006 season were supplied to STC by the industry coordinator. Sub-samples of the seed batches were forwarded to CSL for molecular analysis using TaqMan PCR to test for the presence of *Pfb* DNA on or in the seed. Additionally, growing-on tests on several batches of seed were conducted at STC to investigate any incidence of a seed-borne infection with d. mildew. Batches tested were taken from both the 2005 and 2006 seed. The growing-on test was conducted by sowing 1,000 seed/batch into standard seed compost in multiple seed trays (100/tray) covered with propagator covers. The compost and seedlings were misted regularly with water to maintain conditions suitable for leaf infection to develop. The trays were kept in incubators set to 17ºC with 12hrs lighting/day (Plates 6 & 7).

Plate 6. Seedlings in propagation tray

Plate 7. Seedling test in incubator

Following germination, seedlings were examined daily using a magnifying glass to check for the development of downy mildew infection. Seedlings were monitored for 3 weeks post-germination before being discarded.

For the molecular tests at CSL, 10 DNA extractions were performed each containing 10 seed of each of the various seed-lots provided. The DNA extractions were tested with the *Pfb* TaqMan assay and the quantity of *Pfb* DNA present in or on the seed was determined.

Results

The four sequentially-sown areas of red beet established well in the field at STC during May-June 2006. Whilst the original intention was to inoculate the seedlings at the cotyledon stage this was not possible in the first sowing due to an absence of inoculum of downy mildew in commercial red beet crops. All other inoculation events were carried out as planned using inoculum collected either from field crops in the Isle of Axholme area, or later, using inoculum collected from the non-plot (guard) areas of the trial site at STC where natural wind-borne spread from earlier inoculations had produced suitable leaf infection and sporulating lesions.

Early signs of the onset of d. mildew infection were observed in the inoculated areas of the 1st sowing on the 10th July (20 days post-inoculation). Regular examination and 'tagging' of plants with crown infections was instigated at this time (see Methods and Materials section). Details of the number of plants with crown infections is shown in Table 2.

Table 2. Numbers of plants observed with d. mildew crown infections

Treatment	No. of plants with crown infections in each sowing			
	1st (20.7.06)	2nd (20.7.06)	3rd (4.8.06)	4th (4.8.06)
1. Uninoculated control	0	3	15	3
2. Uninoculated + Fungicides	0	18	12	2
3. Inoculated at cotyledon	0	12	36*	13*
4. Inoculated at 2-4 TL	26*	12*	27*	9*
5. Inoculated at 8-12 TL	1	20^	9	0
6. Inoculated weekly from emergence	24*	4*	15*	0*
7. Inoculated weekly from emergence + fungicides	6*	11*	21*	5*
8. Soil disturbance	0	8	8	5
9. Inoculated + soil disturbance	13*	3*	4*	15*
10. Second drilling	0	4	12	8
11. Inoculated + 2nd drilling	29*	4*	17*	5*

** Plots already inoculated by assessment date*
^ Plots only inoculated 2 days prior to assessment
N.B Each inoculated area contained approximately 100 roots.

In-crop sampling

Samples (4 roots) were collected randomly from each plot in each sowing on 3 occasions following inoculation. The collected root samples were scored for the severity of RMD symptoms using the 0-5 scale shown in Appendix 1 before being forwarded to CSL for molecular testing using TaqMan PCR to quantify the *Pfb* DNA present. Due to financial constraints it was not possible to have all the root samples tested and therefore all the root samples provided were sub-sampled at CSL and held under suitable conditions until the

harvest data was available. This ensured that the limited PCR testing that could be carried out was well–focused to provide the best supporting data for the study.

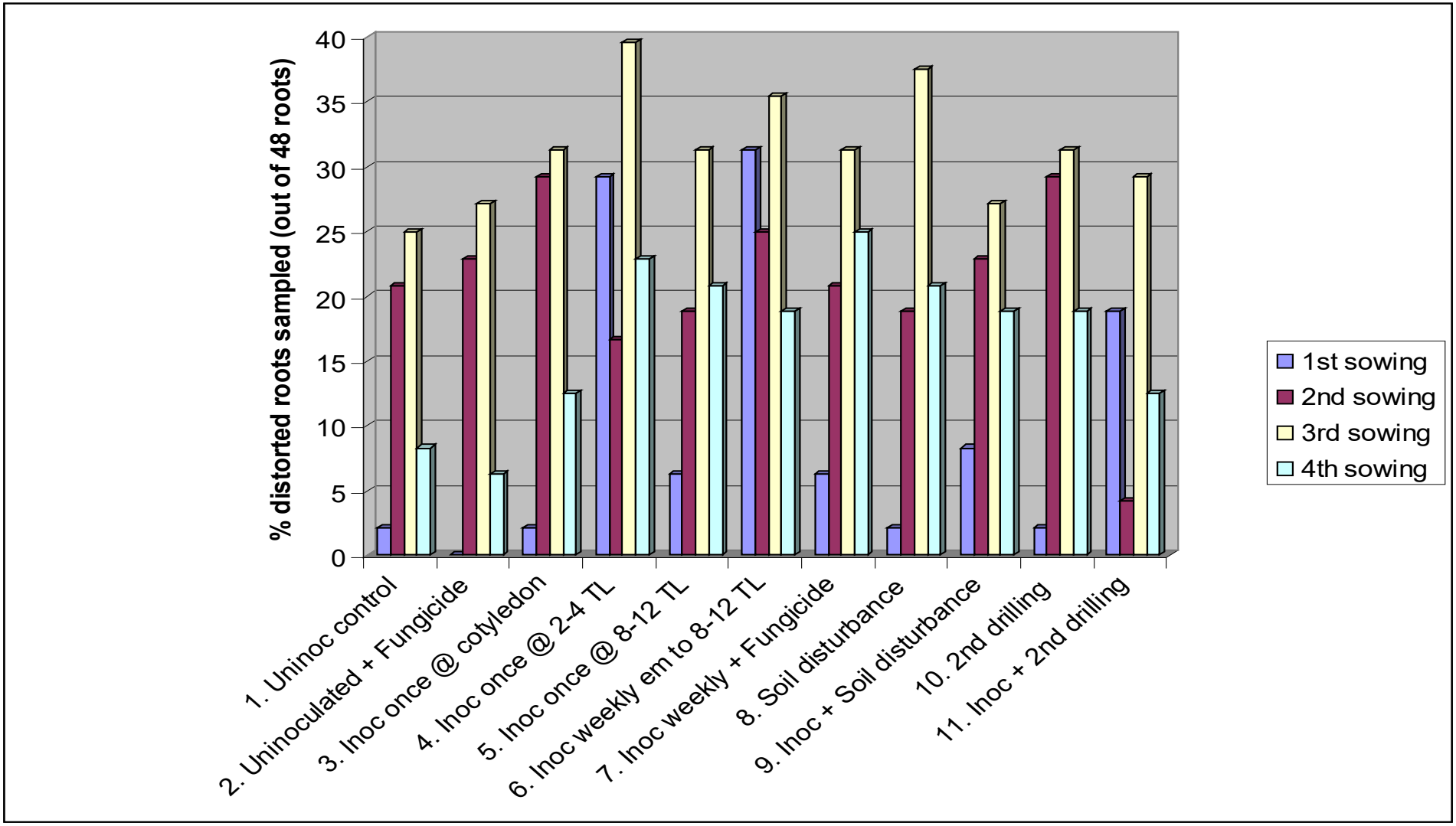
The results of the in-crop RMD assessments are shown graphically (Chart 1). The results for the 1st sowing are of particular interest as they would not have been influenced to the same extent by secondary spread of the pathogen following its initial introduction during inoculation events, compared to the later drilling dates. Here, there was a very low incidence of RMD in the uninoculated control (T1) and an even lower incidence where an oomycete fungicide programme was applied (T2). Yet, inoculation of T4 at the 2-4 leaf stage (note that inoculation with *Pfb* at the cotyledon stage (T3) was omitted due to lack of suitable inoculum in this first drilling date) and T6, which was inoculated repeatedly, showed a high incidence of RMD. Where inoculation with *Pfb* was delayed until the 8-10 true leaf stage (T5) the incidence of RMD was reduced considerably. It is particularly encouraging to note that where a fungicide programme was applied to inoculated plots (T7) the incidence of RMD was significantly reduced.

Taqman PCR analysis of the seedlings in specific treatments (T1, T4, T5, T6 & T7) were also very encouraging as they showed a good correlation between the incidence of RMD and the presence of DNA of the pathogen in the root tissues themselves.

The incidence of RMD in the 4 sowings (Chart 1) indicates that the highest incidence of RMD was observed in the 3rd sowing, whereas the post-harvest results point to the highest occurrence of RMD being observed in the 1st sowing albeit with the 3rd sowing being only slightly behind in terms of incidence and severity of RMD. The reasons for this are not clear. It may suggest that RMD symptoms had yet to develop on roots in the 1st sowing at the time of sampling, but that as the crop matured RMD symptoms became more obvious. Overall the incidence of RMD in the in-crop samples was relatively low, with a lower incidence in the earlier collected samples than in the final sample collected approximately 1 month prior to harvest and this is assumed to be due to the time required for full RMD symptom expression (Full charted values are shown in Appendix 3 where incidence and severity of RMD for each sample time in each sowing are available). In the majority of cases a similar occurrence of RMD is seen across the treatments in each sowing, with less obvious treatment trends.

Chart 1. Mean Incidence of RMD in Random Plant Samples collected during the season

(Data presented as % RMD in sampled roots over 3 sampling dates per treatment)



Once the harvest data was available a decision was made as to which treatments and samples should be subjected to Taqman PCR analysis (due to a budgetary constraint outside our control). Based on the available data, it was concluded that analysis of samples from the first drilling would be most appropriate as they were less influenced by secondary spread of the pathogen via wind-blown sporangia and yet the incidence and severity of RMD was relatively high. The selected treatments for Taqman PCR were as follows:-

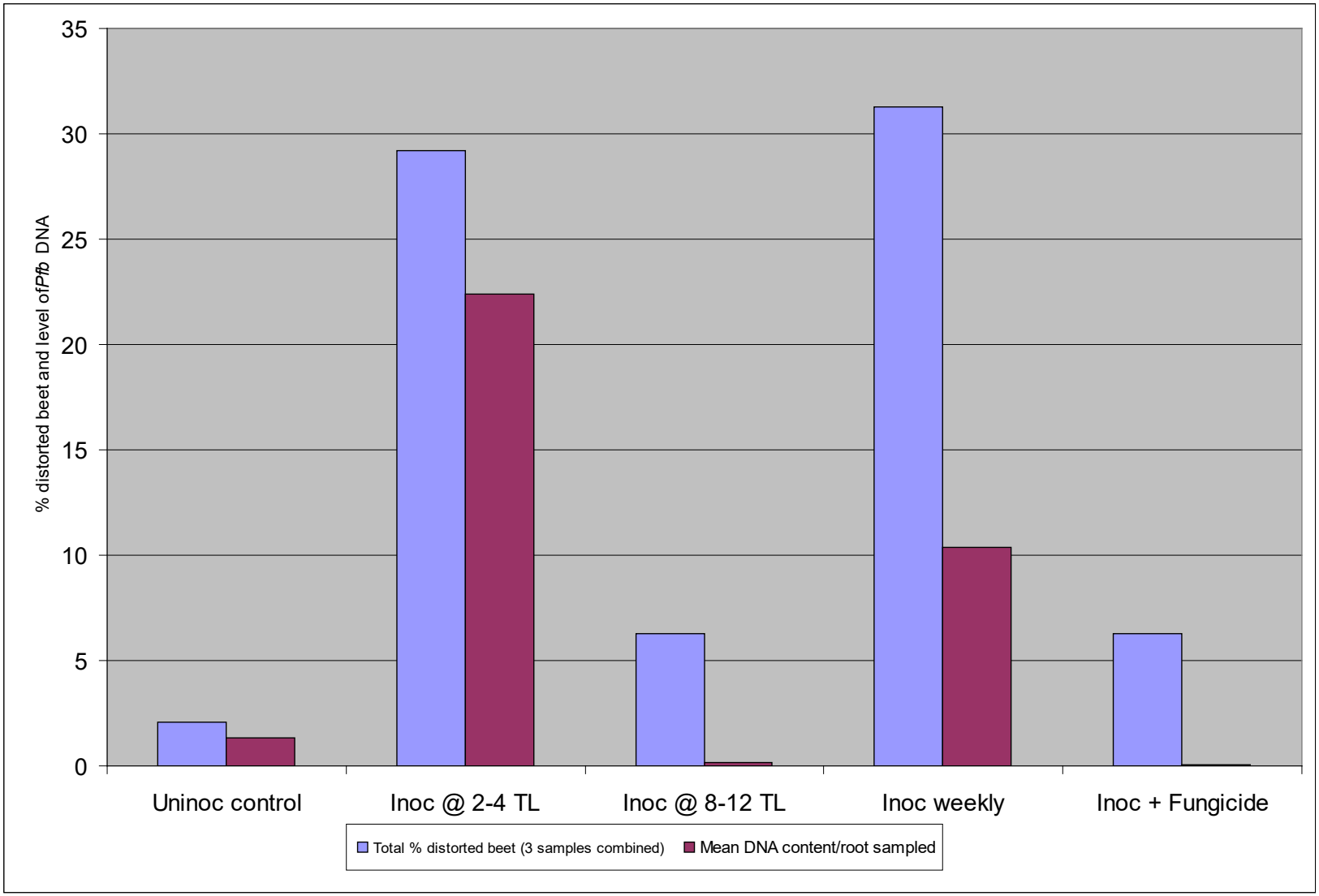
T1 – Uninoculated control – If our hypothesis was correct we would expect to see a relatively low level of *Pfb* DNA here
T4 – Inoculated once at 2-4 true leaf – As there was a high incidence of RMD in this treatment at harvest we ought to find a corresponding high incidence of Pfb DNA; if the hypothesis was correct. (It should be noted that T3 was omitted from the analysis here as it did not receive inoculum in time relative to the crop growth stage)
T5 – Inoculated once at 8-12 true leaf – Here, a lower incidence of RMD was recorded, perhaps signifying a reduced susceptibility to systemic infection as the plants get older. We would therefore expect to see a reduced incidence of Pfb DNA in the sampled plants.
T6 – Inoculated weekly from emergence – The repeated inoculation with sporangia of the downy mildew fungus certainly appeared to give rise to a high incidence and severity of RMD symptoms…but would this also be reflected in a high detection rate of Pfb DNA in the internal root tissues?
T7 – Inoculated weekly + fungicides – The question here was, would the fungicide treatment effectively prevent the inoculum from causing a systemic infection by the downy mildew fungus and hence internal tissue damage leading to RMD? If so, we ought to detect a significant reduction in Pfb DNA here for the hypothesis to be correct.

All the in-crop and post-harvest samples from the selected treatments were sent to CSL for molecular testing.

The results from the in-crop sampling of the 1st sowing of red beet are shown in Chart 2. The chart shows a comparison between the number of distorted beet found (mean of the 3 sampling dates) and the level of *Pfb* DNA detected in the internal root tissues. There is a good correlation between these parameters indicative of cause and effect; and hence supporting the hypothesis that RMD is caused by the downy mildew fungus. A higher incidence of RMD was recorded in plots which were inoculated at the 2-4 true leaf stage (T4) and those which were inoculated weekly from emergence through to the 8-12 true leaf stage (T6) relative to either the uninoculated (T1) samples, those which had been inoculated at the 8-12 true leaf stage (T5) or in the inoculated fungicide treated plots (T7). Importantly, this

correlates closely with the mean *Pfb* DNA content of the roots within each of these treatments. Tabulated data for the remainder of the in-crop sampling can be found in Appendix 2.

Chart 2. Mean Incidence of RMD in Plant Samples from the First Drilling Relative to the Presence of Pfb DNA in the Internal Root Tissues (selected treatments only)

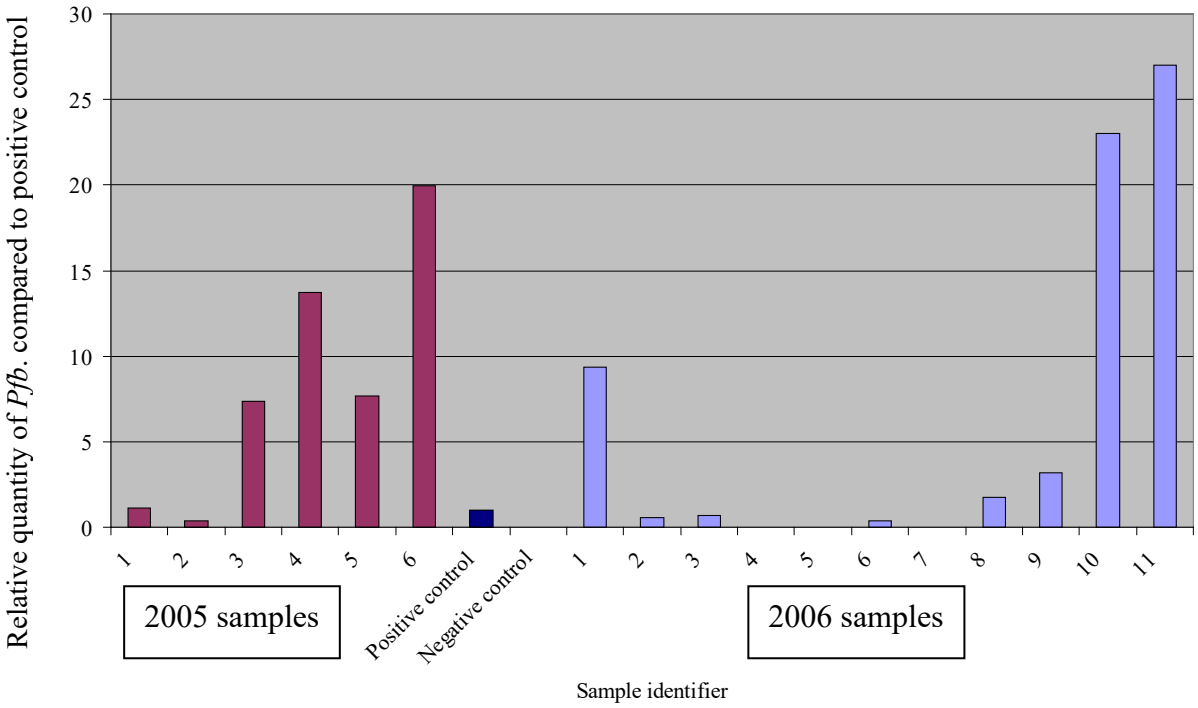


Seed Testing

Seed PCR assay results

The TaqMan PCR assay was used to test 6 batches of seed saved by a commercial grower in 2005 and a further 11 samples from 2006 (Chart 3).

Chart 3. Results of Seed PCR assays



The assay measures the quantity of *Pfb*. DNA present on the seed compared to a positive control (labelled) which has a nominal value of one. Each value shown represents a mean of 10 sub-samples (each sub-sample made up of 10 seed tested as a batch). Whilst the results presented indicate a high level of recovery of *Pfb* DNA in some seed samples in both 2005 & 2006 it is important to recognise that they do not, on their own, signify a seed-borne cause for RMD. It is important to appreciate that, whilst the results potentially give cause for concern, the TaqMan assay merely detects DNA material though is unable to differentiate between viable and non-viable fungal cells.

Seed growing-on tests

A number of seed batches were sown and grown-on as described in the Materials and Methods section in an attempt to further investigate the risk of seed-borne infection with *Pfb*. None of the seed tested in this way resulted in an obvious infection with d. mildew i.e. no sporulation was observed. Some leaf distortion symptoms were recorded, though it was not clear whether the distortion was linked with d. mildew or was the result of damage caused

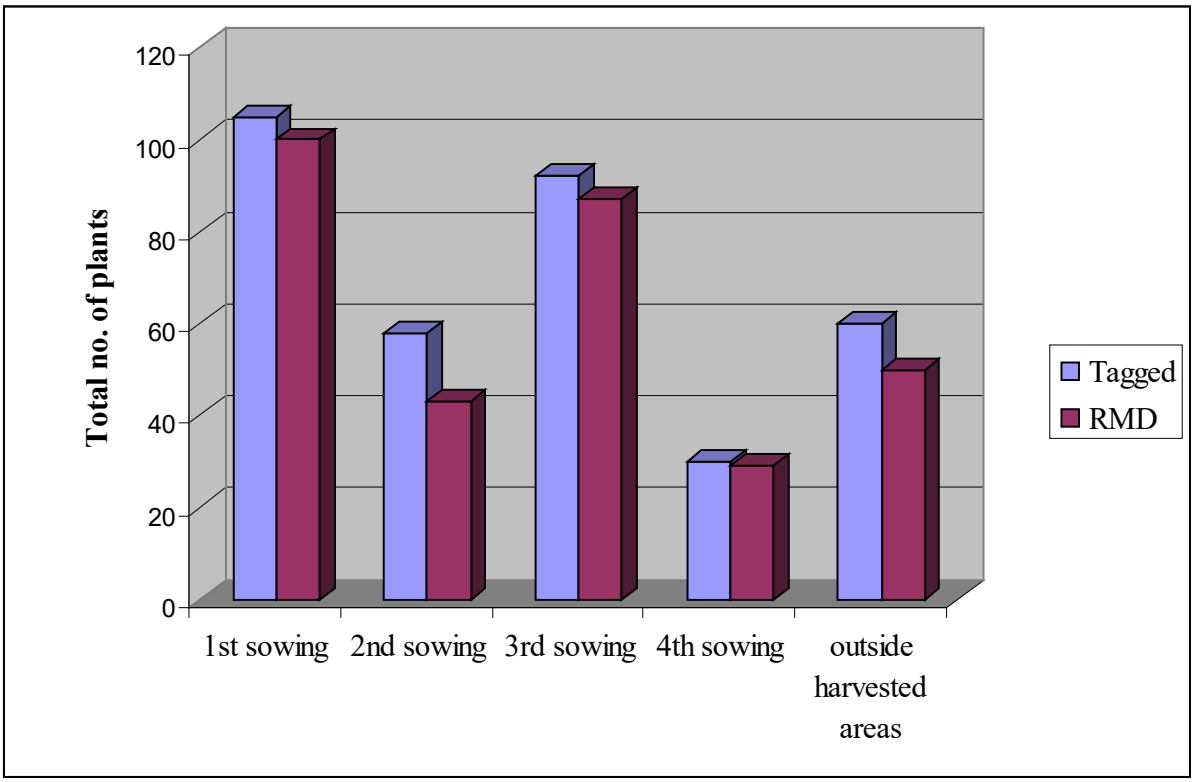
during leaf development through the seed coat. Subsequent PCR analysis of a batch of distorted red-beet seedlings proved negative for the presence of *Pfb* DNA. There was certainly no immediately apparent correlation between leaf distortion in the growing on test and the recovery of *Pfb* DNA in specific seed batches. Discussions with colleagues in other research groups where similar growing-on tests are conducted suggests that this type of assay is inherently unreliable. It is necessary therefore to find an alternative approach to confirm whether the PCR analyses of the different seed batches is significant from a disease risk perspective.

Harvest Results

The inoculated, treated and replicated plots in each of the 4 trial areas (sowings) were harvested between October 2006 and January 2007. All of the beet (4 rows) in each plot (1.8m x 0.8m) were lifted. The total number of beet/plot was recorded along with the number and severity of any beet with RMD symptoms (Table 3 & Charts 5 & 6). The full assessment data is presented in Appendix 4).

A record was also made of any roots harvested in this area which had been previously 'tagged' to enable us to monitor correlations between observed d. mildew crown infections and RMD development. These were recorded on plants which had been tagged within the inoculated areas or in corresponding areas in uninoculated plots (Chart 4).

Chart 4. Tagged plants with (systemic) crown infection caused by downy mildew and the incidence of RMD symptoms on the same plants at maturity



On average, 89% of the plants which had been tagged to signify crown infection (systemic) with downy mildew subsequently developed RMD symptoms on the roots and this signifies a very strong correlation between the two symptoms (this correlation has also been seen in tagged commercial crops carried out within this study area in previous years). This data undoubtedly helps confirm cause and effect between downy mildew and the RMD symptom.

Plate 8. Harvested non-distorted (top) roots and roots showing distortion consistent with those of RMD (bottom).

Table 3. Incidence and severity of RMD at harvest in the sequentially drilled crops of Red Beet in Autumn 2006

Treatment	1st Sowing*		2nd Sowing		3rd Sowing		4th Sowing	
	Mean % beet with RMD/plot	Mean RMD severity/plot (0-100 index)	Mean % beet with RMD/plot	Mean RMD severity/plot (0-100 index)	Mean % beet with RMD/plot	Mean RMD severity/plot (0-100 index)	Mean % beet with RMD/plot	Mean RMD severity/plot (0-100 index)
1. Uninoculated control	7.8^{c}	3.5^{c}	7.6^{abc}	4.5^{ab}	5.3^{c}	2.4^{c}	2.2^{ab}	1.4^{ab}
2. Uninoculated + fungicides	6.2^{c}	2.7^{c}	0.5^{c}	0.3^{b}	3.3^{c}	0.9^{c}	0.2^{b}	0.2^{b}
3. Inoculated : cotyledon stage	5.0^{c}	2.5^{c}	18.0^{ab}	10.9^{ab}	30.0^{ab}	19.5^{b}	3.0^{ab}	1.2^{ab}
4. Inoculated : 2-4 leaf stage	35.0^{a}	26.8^{ab}	19.1^{a}	13.8^{a}	26.7^{b}	16.8^{b}	1.8^{ab}	0.6^{ab}
5. Inoculated : 8-12 leaf stage	12.8^{bc}	8.2^{c}	11.6^{abc}	5.9^{ab}	11.0^{c}	4.8^{c}	3.2^{ab}	1.7^{ab}
6. Inoculated weekly	39.9^{a}	31.7^{a}	17.7^{ab}	10.9^{ab}	36.8^{a}	24.7^{a}	5.8^{a}	3.3^{a}
7. Inoculated weekly + fungicide	17.7^{bc}	11.3^{c}	1.5^{bc}	1.0^{b}	5.0^{c}	3.6^{c}	0.7^{b}	0.5^{ab}
8. Soil disturbance	8.6^{c}	4.2^{c}	13.5^{abc}	8.3^{ab}	8.6^{c}	4.7^{c}	4.0^{ab}	2.3^{ab}
9. Inoculated + soil disturbance	26.2^{ab}	17.5^{bc}	13.8^{abc}	8.9^{ab}	7.2^{c}	4.2^{c}	3.3^{ab}	2.2^{ab}
10. Second drilling	8.9^{c}	4.5^{c}	12.4^{abc}	8.2^{ab}	6.8^{c}	3.0^{c}	3.7^{ab}	2.1^{ab}
11. Inoculated + second drilling	35.3^{a}	24.1^{ab}	13.0^{abc}	9.5^{ab}	10.8^{c}	5.7^{c}	1.4^{ab}	1.1^{ab}
LSD (P=0.05)	11.3	10.3	10.3	6.7	6.9	5.0	2.9	1.7
Std. Dev.	7.8	7.1	7.2	4.7	4.8	3.5	2.0	1.2
Coefficient of Variance	42.5	57.1	61.3	62.6	34.8	42.2	76.6	80.4

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

* It is important to note that seedlings in the first drilling could not be inoculated at the cotyledon stage (T3) due to the absence of *Pfb* inoculum at this time.

The data collected at harvest in Autumn 2006 finally provided the evidence needed to confirm the hypothesis that RMD is caused by the downy mildew fungus. This is the first time that RMD symptoms have been reproduced experimentally in red beet.

In the 1st sowing (where the issue of secondary spread of *Pfb* inoculum was of less concern) a significantly higher incidence of RMD developed when the seedlings were inoculated early (T4, 6, 9 and 11) compared to those which had either not been inoculated (including T3 as no inoculum was available at this particular growth stage at the first drilling date) or those which had been inoculated but fungicide treated. In some plots the incidence of RMD exceeded 60% of the harvested roots. It was also interesting to observe that seedlings inoculated later at the 8-10 true leaf stage (T5) the incidence of RMD was much reduced indicating, perhaps, that beet seedlings become less susceptible to systemic infection with *Pfb* as they become older. It also implies, therefore, that red beet crops are most vulnerable to systemic infection by *Pfb* at the young seedling stage and this perhaps provides a pointer to the optimum timing of fungicides for effective control. A high incidence and severity of RMD was also observed in the inoculated plots of the soil disturbance and 2nd drilling treatments (T9 & 11). However, there is no evidence from the data to suggest that these treatments exacerbated the level of RMD relative to the inoculated control.

In the 2nd sowing, whilst the overall severity of the distortion was lower, the highest incidence and severity of infection was observed in T3 and T4 where the plants had been inoculated at an early stage in their development (cotyledon and 2-4 TL respectively). This observation supported that found in the first drilling and could prove to be of considerable significance to the red beet industry with respect to optimised timing of fungicide application. However, further work is required in different seasons to confirm this observation. Relative to the uninoculated control (T1), there was a moderately high incidence of RMD in most treatments though where an oomycete fungicide programme was applied it provided effective control of RMD and this is particularly encouraging for growers. The reduced incidence and severity of RMD and the number of tagged plants with crown infections (Chart 4) in the 2nd drilling, relative to that recorded in the 1st drilling, is considered to be due, to a large part, to the prevailing weather at the time being less conducive to systemic infection. However, the precise parameters required for systemic invasion of the hypocotyl by this fungal pathogen are not known and further work would be required to define this. Interestingly, a higher incidence of foliar downy mildew infection (Plate 8) was apparent in this 2nd drilling. We believe this was due to natural transmission from the adjacent highly infected 1st drilling of red beet. It suggests, perhaps, that the optimum conditions for foliar infection may be slightly different to those for systemic infection of the hypocotyl. It is, of course, possible that the

extensive sporangial production from leaf infection could, under suitable wet weather conditions, be washed down into the crown region to initiate systemic infection; though this has not been confirmed in this work.

Plate 9. Downy mildew foliar infection on red beet

In the 3rd sowing *Pfb* inoculation at the cotyledon, 2-4 true leaf stage and weekly inoculations from emergence (T3, 4 and 6) all resulted in a significantly higher incidence and severity of RMD compared to the remaining treatments. In comparison, the equivalent treatments which received fungicide applications (T2 and T7) had a low incidence and severity of RMD infection, despite the early and repeated inoculations with d. mildew. This demonstrated the potential efficacy of an oomycete fungicide programme in reducing crown and foliar *Pfb* infections and ultimately RMD symptoms at harvest.

The final sowing was harvested on the 10th January. The crop was noticeably smaller with few beet larger than baby beet size, suggesting that the late sowing date (16.6.06) was too late for commercial purposes. The incidence and severity of RMD in this crop was the lowest overall (<6%) with the highest incidence recorded in T6 which had been inoculated weekly from emergence until the plants had reached the 8-12 true leaf stage. RMD severity was markedly lower in the affected beet throughout. Where oomycete fungicides were applied to equivalent inoculated plots (T7) the incidence of distortion was < 1% with a mean severity of 0.5. This data amply demonstrates that it is not merely the growth stage of the crop that dictates susceptibility to downy mildew infection and RMD. In this late sown crop the weather conditions, particularly temperature, were evidently less conducive to infection (even though the crop appeared susceptible) and hence infection of the seedlings and young plants was significantly reduced.

Chart 5. The incidence of RMD in the harvested areas of the four sowings

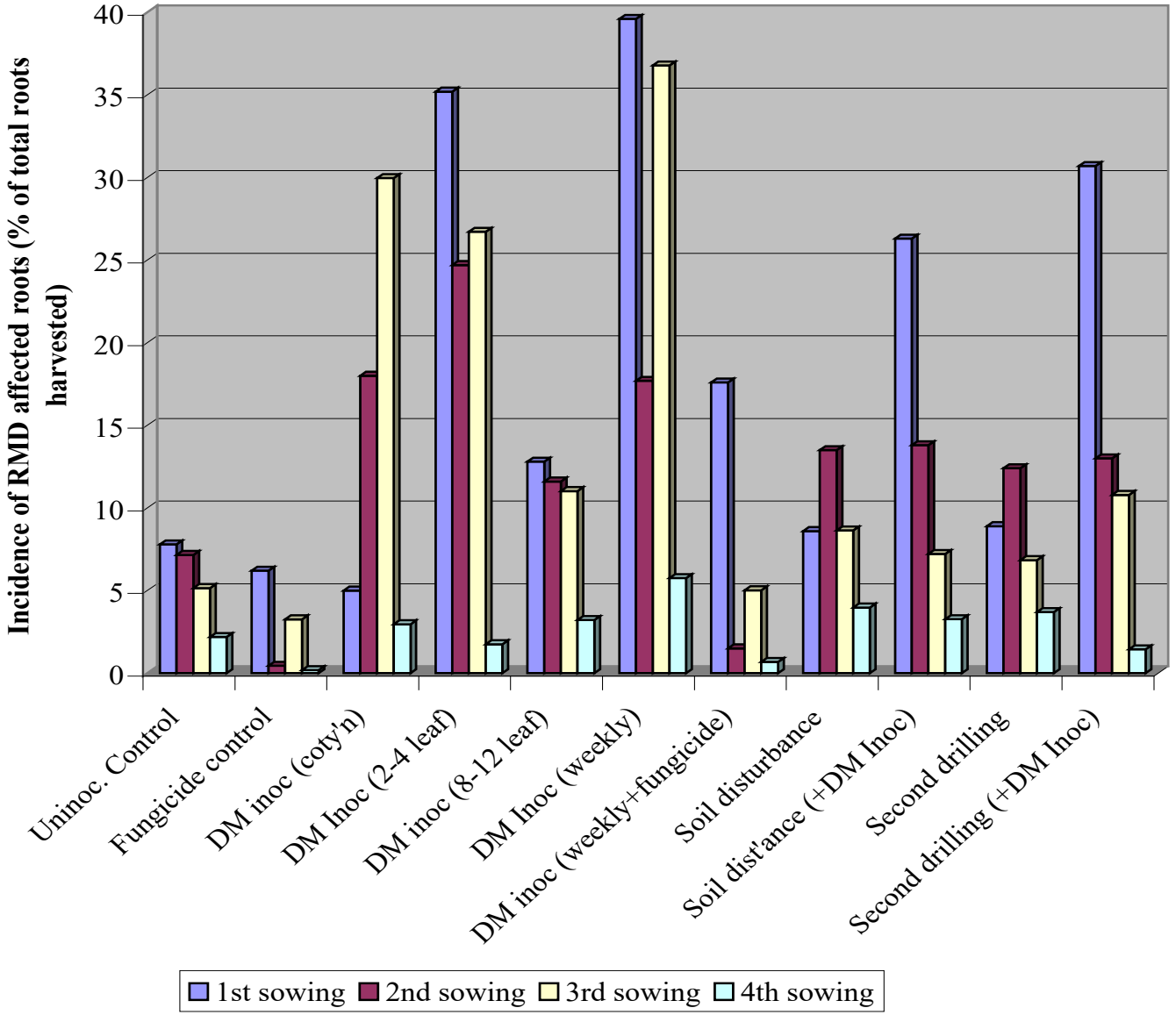
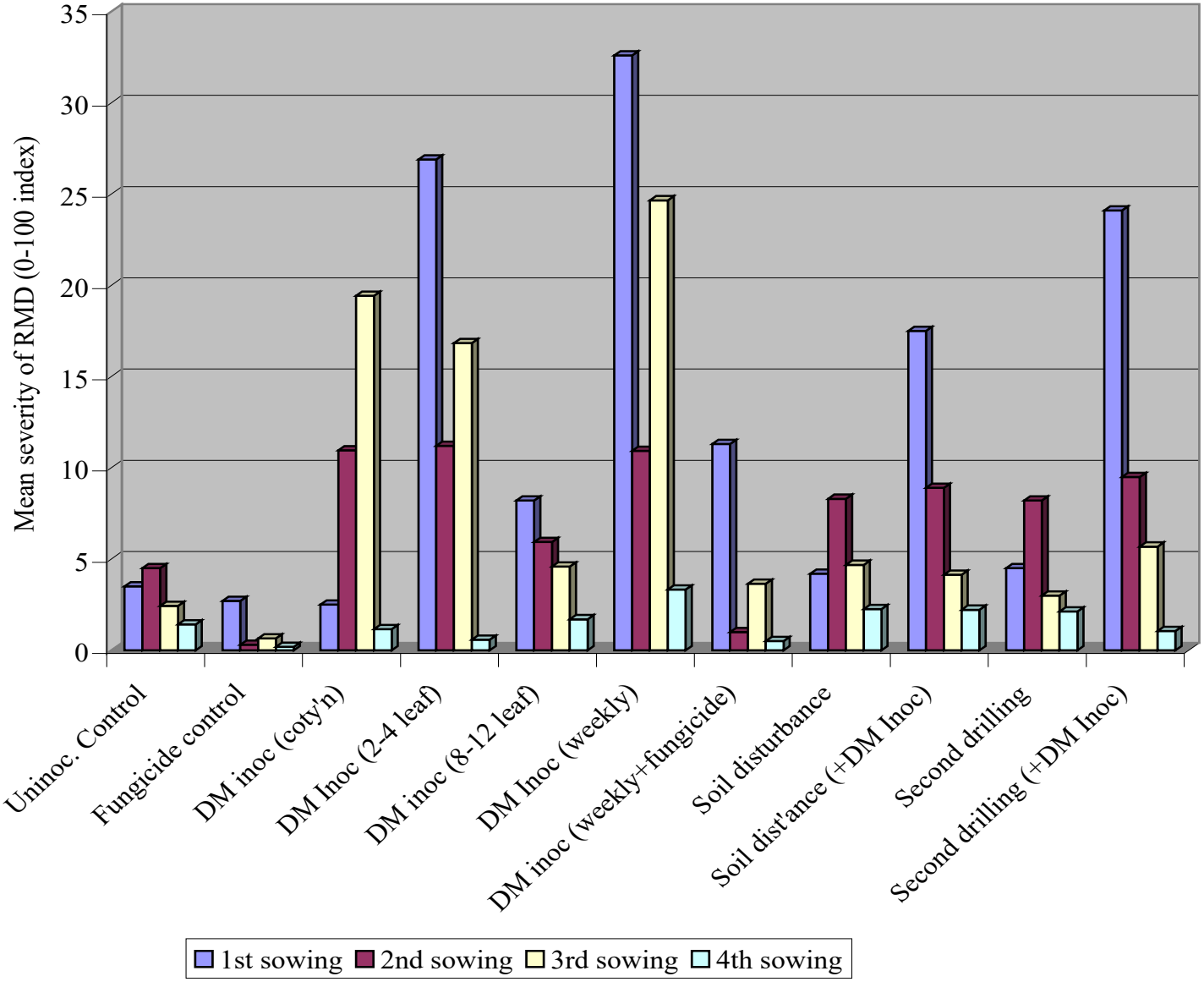


Chart 6. The severity of RMD in the harvested areas of the four sowings



At maturity, a number of distorted and healthy beet were retained for further molecular testing. As with the in-crop (seedling) samples, budgetary constraints limited the number of samples which could be tested by TaqMan PCR and a decision was made to test post-harvest samples from the same sowing and treatments as used for the seedling tests i.e. T1, T4, T5, T6 and T7 in the first drilling.

Whilst the results of the PCR testing on the harvested root samples proved to be somewhat variable, overall they supported the hypothesis that RMD is caused by the downy mildew pathogen *P. farinosa* f.sp. *betae*

Discussion

Experimental work carried out at STC in 2006 has finally enabled us to reproduce RMD symptoms in red beet experimentally following field inoculation with the downy mildew fungus *Peronospora farinosa* f.sp. *betae.* Molecular Taqman PCR analyses have been used to substitute conventional isolation techniques to successfully demonstrate cause and effect. We can therefore finally confirm the previously raised hypothesis that the primary incitant of RMD is the downy mildew fungus; the RMD symptom probably arising as a direct result of a systemic invasion of the hypocotyl and internal cellular damage during the establishment phase of the crop.

Four sequential sowings of red beet cv Crimson Globe were established successfully at STC in late Spring 2006. After an initial delay we were able secure some active downy mildew inoculum from a commercial source (a red beet crop in the Isle of Axholme) despite the relatively high spring temperatures and regular fungicide applications being applied by some growers. The *Pfb* inoculum was 'harvested' and used to inoculate relevant plots in the early sown red beet trial plots. A moderate level of infection by downy mildew was first observed in the inoculated plots some 20 days later. The level of infection increased rapidly, largely due to the regular irrigation of the crop (3 times/week for the duration of the trial) which maintained leaf wetness and high humidity beneath the leaf canopy which encouraged both the infection process and subsequent disease development. Due to the good establishment of the pathogen in the experimental area and its natural transfer into adjacent guard areas we were able to subsequently use this inoculum for later inoculation events. However, the localised dissemination of the pathogen prior to artificial inoculation of plots as per the prescribed schedule, whilst anticipated, did result in a slight 'muddying of the waters' in terms of clear-cut results between inoculated and uninoculated plots. This was considered an acceptable, and largely unavoidable, consequence of this type of work with an air-borne foliar pathogen.

The incidence and severity of RMD was recorded in the experimental plots during in-crop sampling events throughout the growing season and also at each harvest date. The highest occurrence and severity of RMD symptoms was observed in roots harvested from the 1st sowing with values recorded following harvest of the 3rd sowing following closely behind. Lower levels of RMD were seen in the 2nd and 4th sowing of red beet during this season. This, we believe, was due to the variable weather conditions experienced during summer 2006.

Of particular interest was the fact that higher levels of RMD were recorded in treatments which were inoculated when the seedlings were very young. This suggests that the seedlings are particularly vulnerable to systemic invasion by the downy mildew pathogen, presumably following leaf infection, from the cotyledon to the 4 true leaf stage. The results show that

seedlings which were inoculated later (at the 8-12 true leaf stage), whilst still being vulnerable to leaf infection, had fewer and less severe RMD symptoms in comparison. Whilst further work is required to validate this observation and demonstrate that it is not merely a reflection of the 2006 season, it does provide a strong pointer for growers looking to improve the timing of fungicides for RMD control.

It is also very pertinent, that the fungicide programme applied in this study (Fubol Gold + Invader) markedly reduced the levels of RMD and this will provide the industry with a greater level of confidence in formulating spray programmes for RMD control in the future. However, it should be noted that the use of dimethomorph (Invader) is currently not approved for the use on red beet, though efforts are being made to secure it for resistance management purposes.

Symptom expression was very low across all the treatments in the 4th sowing. This strengthens observations and conclusions from the trials that seedling age and timing of infection were critical factors in the systemic development of downy mildew crown and foliar infections and the subsequent development of RMD symptoms on roots. However, as indicated previously, it is important to recognise that this work was conducted over a single season and it is therefore difficult to fully differentiate between genuine treatment effects and seasonal influences. It is recommended therefore that until further information is available with respect to seedling age and susceptibility to downy mildew growers should 'err on the side of caution' and maintain fungicide protection throughout the main growing period of the crop.

The Taqman PCR analyses on seed batches as used in the 2005 & 2006 cropping years were interesting as they appear to suggest that some seed batches appear to be heavily contaminated with *Pfb* DNA (> 25 times the positive control in one case) and this might imply a seed-borne disease risk with the downy mildew pathogen in red beet. If this were the case it could, potentially, account for the unusual and sporadic occurrence of RMD in 1998 and 2002, especially if the prevailing weather conditions were also conducive to infection. However, due to the nature of the assay, i.e. that it is unable to distinguish between DNA from viable or dead cells, the results cannot be considered as positive evidence of seed-borne transmission, especially as infection was not detected in 'growing-on' bioassays using the same seed under controlled conditions in the laboratory. It must be noted however that the growing-on test in itself is not robust and other workers have reported difficulties in achieving infection and symptom expression with d. mildew pathogens in this test. It is therefore recommended that further work is carried out to thoroughly investigate and evaluate any possible seed-borne risk with Pfb. It is hoped that the various seed producers would be fully supportive of this type of work; the aim

being to ensure beet seed was ‘fit for purpose’ and didn’t carry a risk of seed-borne downy mildew.

Finally, whilst the results of this investigation do finally prove conclusively that RMD is caused by a systemic infection by *Peronospora farinosa* f.sp. *betae*, it must be remembered that the conclusions drawn regarding possible high risk growth stages, the efficacy of fungicides and the impact of various environmental parameters are all based on a single experiment in one year, and therefore some care should be taken in interpreting these results especially where they are to be used for commercial practice.

Conclusions

- A complex series of fully replicated field trials involving four sequentially sown crops of red beet were carried out at STC in 2006.

- Classic symptoms of root malformation disorder (RMD) were successfully reproduced for the first time following regular and repeated inoculation with a spore suspension of the downy mildew pathogen *Peronospora farinosa* f. sp. *betae (Pfb)*.

- A significantly higher incidence of RMD was observed where red beets were inoculated with sporangia of *Pfb* as compared to uninoculated beet.

- Where an oomycete fungicide programme was applied to *Pfb* inoculated beet effective control of both downy mildew leaf infection and RMD was achieved.

- A strong correlation was found between the incidence of crown infection by d. mildew and the subsequent RMD development in 'tagged' plants and this supports previous observations in commercial field crops.

- Downy mildew (*P. farinosa* f.sp. *betae*) was generally present at low to negligible levels in commercial red beet crops during 2006. This is considered to be due, in part; to the generally unfavourable weather conditions for infection but also to the increased use of Wakil treated seed and in-crop oomycete fungicide treatments.

- Root distortion (RMD) levels were also very low in commercial beet crops at harvest in Autumn 2006. Reports from growers suggest that typically <1% of graded roots were affected. This observation correlates well with the low-negligible level of downy mildew leaf infection found in crops.

- DNA of *P. farinosa* was detected at high but variable levels on 11 batches of seed using the TaqMan PCR assay. However, as the test cannot differentiate between DNA from viable and non-viable propagules the data needs to be treated with caution. Growing-on tests using the same seed batches failed to express characteristic downy mildew infections

either though, information from other workers, suggests that the growing-on test method is not entirely robust, and therefore the results are considered to be generally inconclusive. It is

recommended that further work is conducted in the future to provide a definitive answer regarding the risk of seed-borne downy mildew infection and subsequent systemic RMD development in red-beet.

Technology Transfer

As in previous years the information from this project has been relayed to the industry throughout the season via the Red Beet Technology Group meetings, in one-to-one contact with growers and the various activities of both the HDC Project Co-ordinator and the project team.

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

A presentation and discussion of the results of this investigation was made to members of the Red Beet Technology Group at their annual meeting on the 15th February 2007 at STC.

Using this HDC funded project on red beet as an example of 'Science into Practice' STC made an application to the Grower of the Year Awards in March 2007. The submission succeeded to the second round and STC finally won the award for 'Science into Practice' at a prestigious event in London. The award was accepted by Dr McPherson who acknowledges the efforts of everyone in the research team for their efforts in resolving the RMD enigma with special thanks to Graham Smith who fiercely defended his industry and assisted in securing the HDC funding which allowed us to undertake the necessary work to solve the problem.

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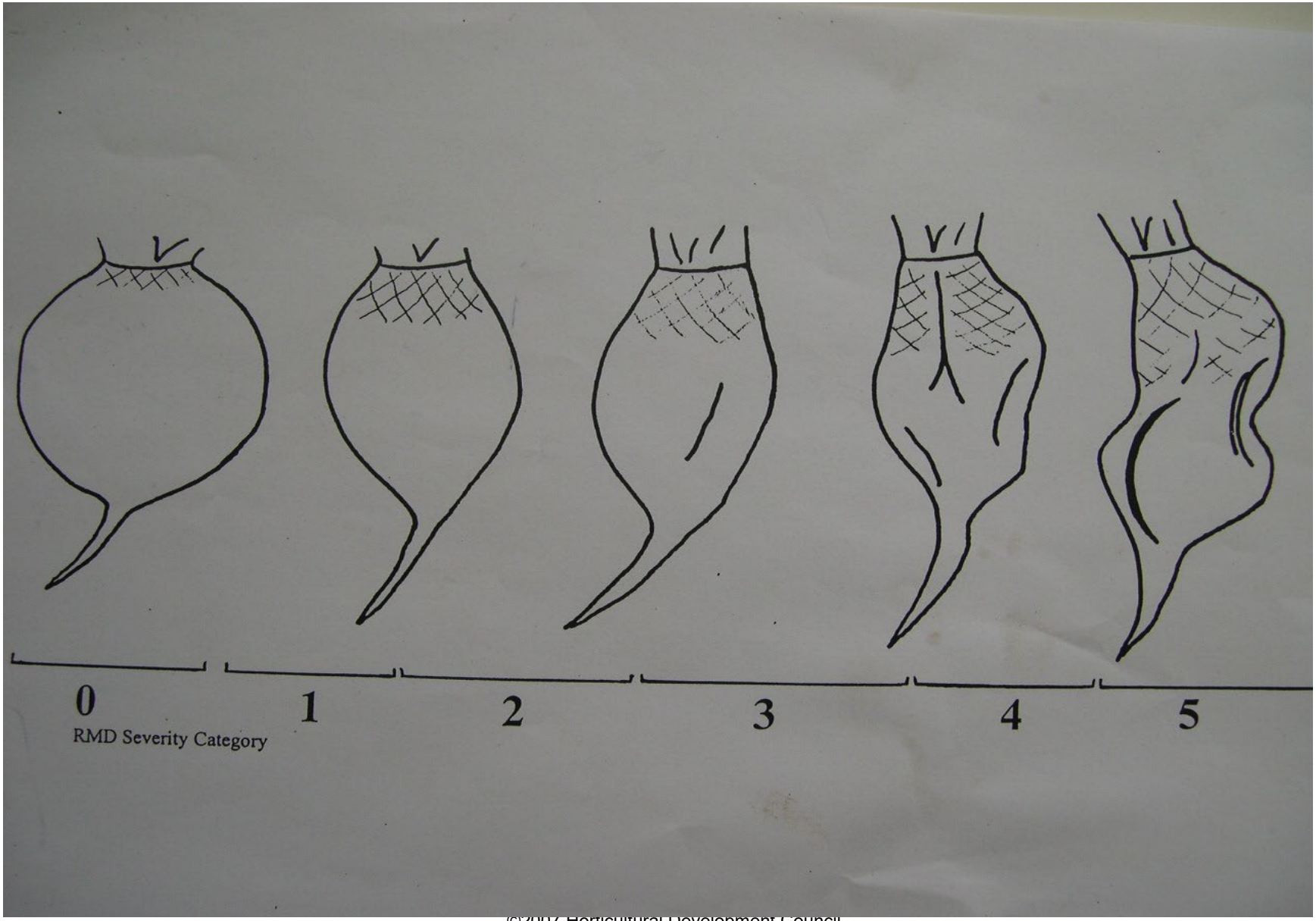
Acknowledgements

Our thanks and grateful appreciation goes to Graham Smith at South Carr Farm for his continued enthusiasm, help, support and advice during the field phase of this trial. We are also grateful to Neil Boonham, Kathy Walsh and Rachel Glover at the Central Science Laboratory, York, for carrying out the molecular analyses on the red beet and seed samples throughout the season. Thanks to Elsoms Seeds for providing the Crimson Globe seed for the field trial work. Finally, thanks to the many growers, consultants and trade representatives who have helped in one way or another to aid resolution to the on-going problem of RMD in the red beet crop.

Appendix 1- 0-5 Severity assessment scale for RMD symptoms



Appendix 2. Tabulated data of the incidence and severity of RMD found in the samples collected during the growing period.

1st Sown Block

Treatment	1st sampling date		2nd sampling date		3rd sampling date	
	No. of distorted beet	Mean RMD severity 0-100 index	No. of distorted beet	Mean RMD severity 0-100 index	No. of distorted beet	Mean RMD severity 0-100 index
1. Uninoc control	0	0.0	1	1.3	0	0.0
2. Fungicide uninoc	0	0.0	0	0.0	0	0.0
3. Inoc once at cotyledon	0	0.0	1	1.3	0	0.0
4. Inoc once at 2-4 TL	4	11.3	7	17.5	3	8.8
5. Inoc once at 8-12 TL	0	0.0	1	1.3	2	3.8
6. Inoc weekly	5	12.5	5	13.8	5	21.3
7. Inoc weekly + fungicide	0	0.0	1	1.3	2	2.5
8. Soil disturbance	0	0.0	0	0.0	1	1.3
9. Soil disturbance + inoculation	1	2.5	1	1.3	2	3.8
10. 2nd drilling	0	0.0	1	1.3	0	0.0
11. 2nd drilling + inoculation	2	5.0	3	5.0	4	15.0

2nd Sown Block

Treatment	1st sampling date		2nd sampling date		3rd sampling date	
	No. of distorted beet	Mean RMD severity 0-100 index	No. of distorted beet	Mean RMD severity 0-100 index	No. of distorted beet	Mean RMD severity 0-100 index
1. Uninoc control	0	0.0	3	13.8	7	8.9
2. Fungicide uninoc	1	1.3	3	7.5	7	13.8
3. Inoc once at cotyledon	1	2.5	5	11.3	8	20.0
4. Inoc once at 2-4 TL	1	2.5	4	12.5	3	8.8
5. Inoc once at 8-12 TL	1	2.5	1	1.3	7	8.8
6. Inoc weekly	2	2.5	3	3.8	7	12.5
7. Inoc weekly + fungicide	1	1.3	2	11.3	7	11.3
8. Soil disturbance	0	0.0	2	6.3	7	10.0
9. Soil disturbance + inoculation	1	1.3	2	3.8	8	18.8
10. 2nd drilling	2	2.5	2	6.3	10	21.3
11. 2nd drilling + inoculation	1	1.3	0	0.0	1	1.3

3rd Sown Block

Treatment	1st sampling date		2nd sampling date		3rd sampling date	
	No. of distorted beet	Mean RMD severity 0-100 index	No. of distorted beet	Mean RMD severity 0-100 index	No. of distorted beet	Mean RMD severity 0-100 index
1. Uninoc control	1	1.3	2	2.5	9	22.5
2. Fungicide uninoc	2	2.5	2	3.8	9	13.8
3. Inoc once at cotyledon	2	3.8	3	8.8	10	27.5
4. Inoc once at 2-4 TL	2	5.0	8	31.3	9	37.5
5. Inoc once at 8-12 TL	1	1.3	4	6.3	10	23.8
6. Inoc weekly	1	1.3	5	16.3	11	50.0
7. Inoc weekly + fungicide	1	2.5	3	11.3	11	16.3
8. Soil disturbance	2	2.5	3	8.8	13	25.0
9. Soil disturbance + inoculation	2	2.5	1	1.3	10	26.3
10. 2nd drilling	2	2.5	4	10.0	9	15.0
11. 2nd drilling + inoculation	2	2.5	3	3.8	9	13.8

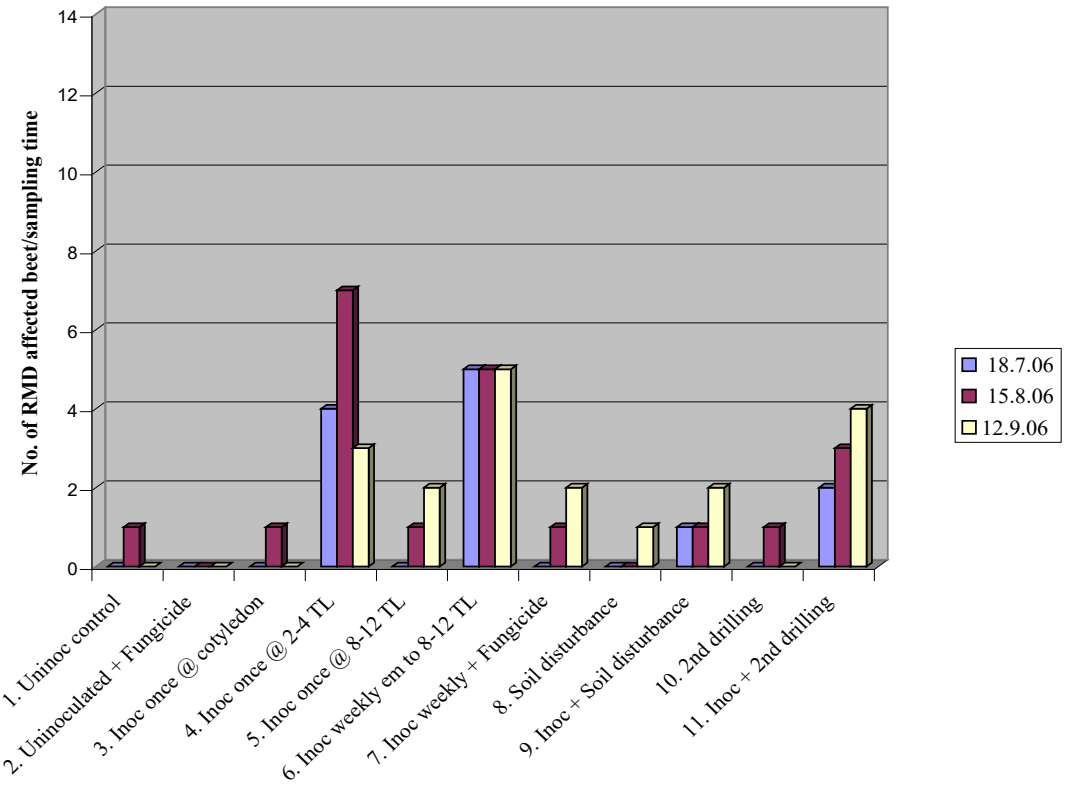
4th Sown Block

Treatment	1st sampling date		2nd sampling date		3rd sampling date	
	No. of distorted beet	Mean RMD severity 0-100 index	No. of distorted beet	Mean RMD severity 0-100 index	No. of distorted beet	Mean RMD severity 0-100 index
1. Uninoc control	0	0.0	2	5.0	2	3.8
2. Fungicide uninoc	0	0.0	0	0.0	3	6.3
3. Inoc once at cotyledon	1	1.3	1	1.3	4	5.0
4. Inoc once at 2-4 TL	1	1.3	6	8.8	4	6.3
5. Inoc once at 8-12 TL	1	1.3	3	5.0	6	12.5
6. Inoc weekly	1	1.3	3	3.8	5	10.0
7. Inoc weekly + fungicide	4	5.0	4	5.0	4	5.0
8. Soil disturbance	1	1.3	2	2.5	7	12.5
9. Soil disturbance + inoculation	0	0.0	3	3.8	6	8.8
10. 2nd drilling	1	1.3	3	10.0	5	7.5
11. 2nd drilling + inoculation	0	0.0	3	6.3	3	5.0

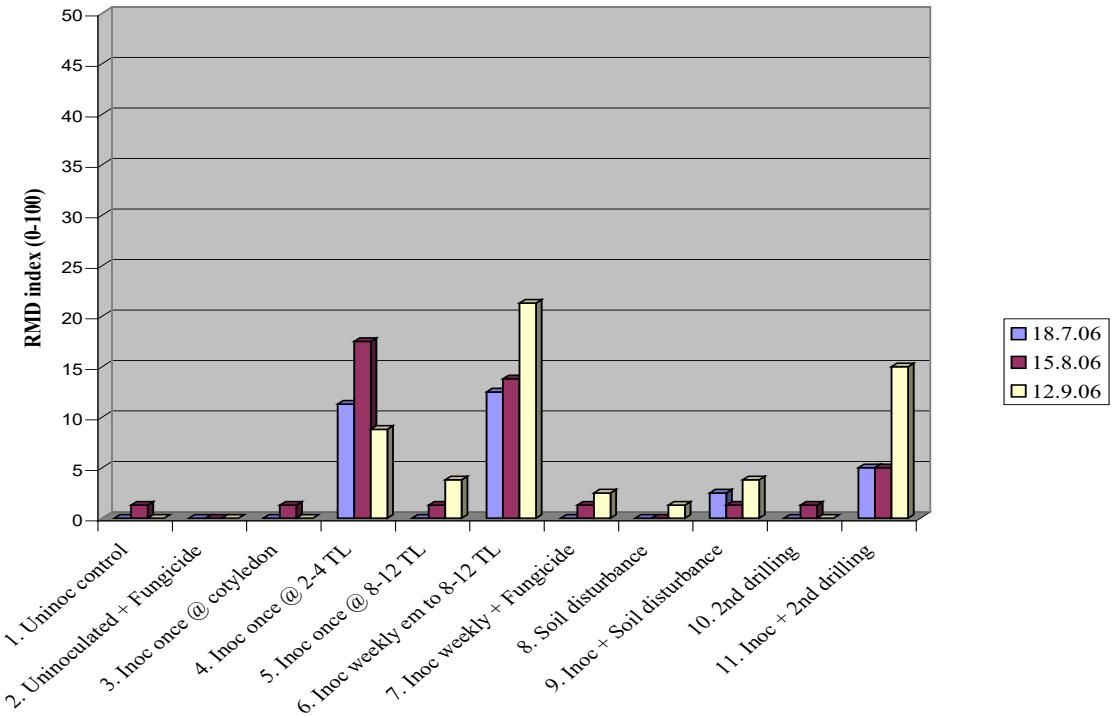
Appendix 3

Charts showing results visual assessments of in-crop sampling for RMD

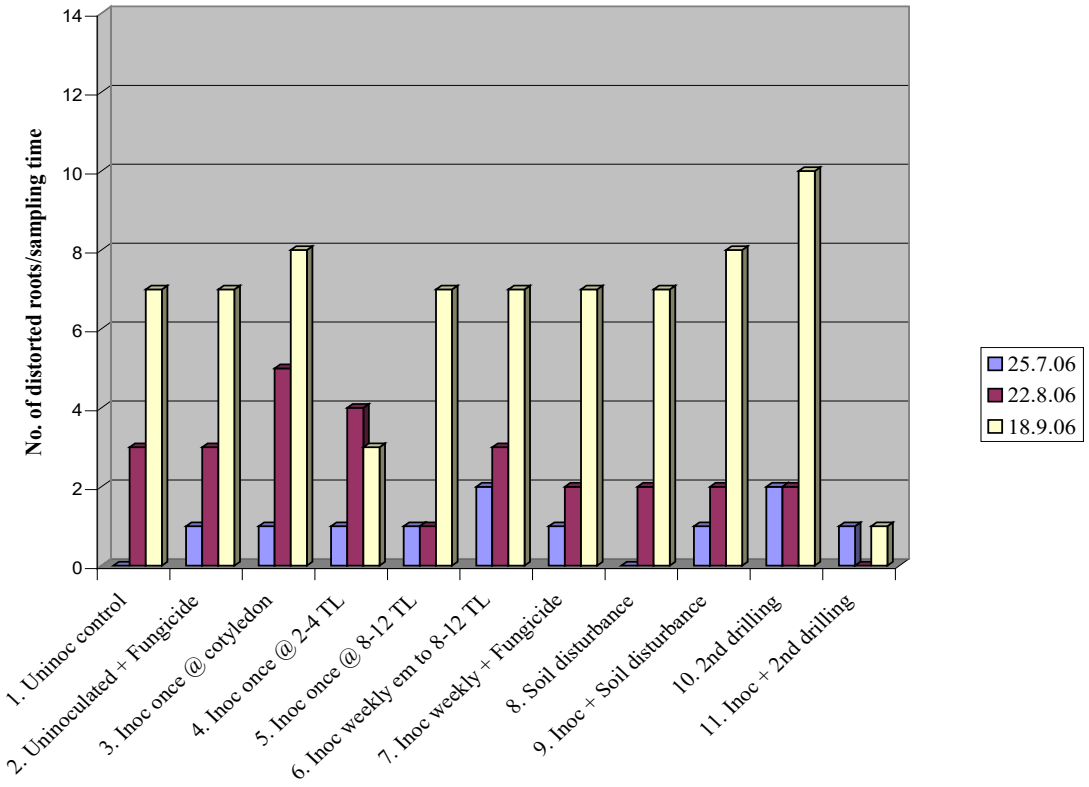
RMD incidence in Sowing 1



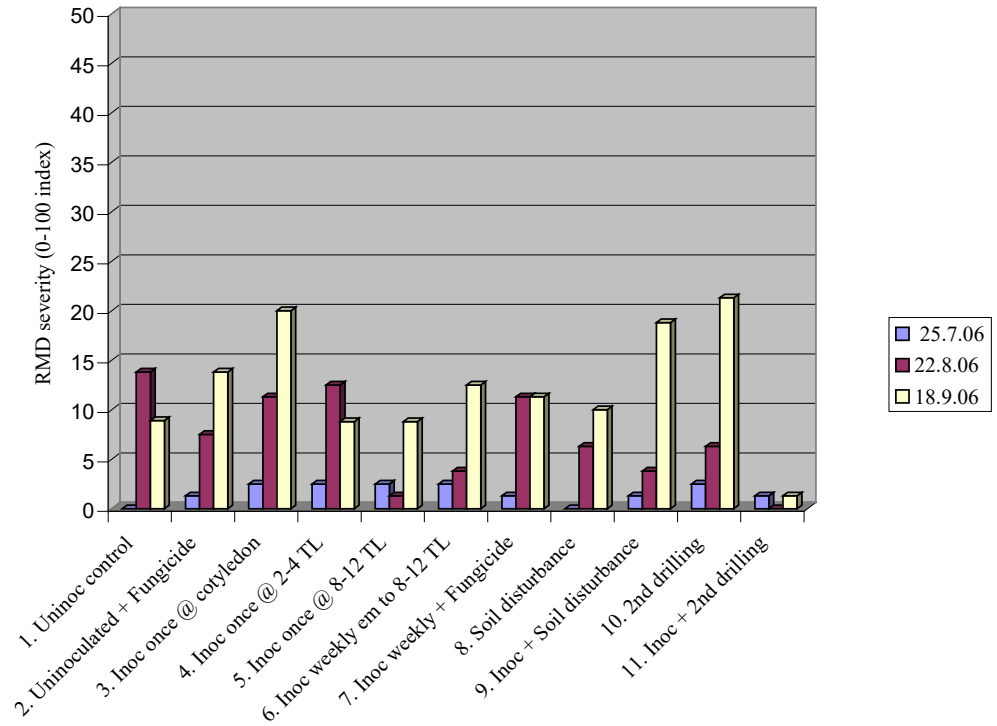
RMD severity in Sowing 1.



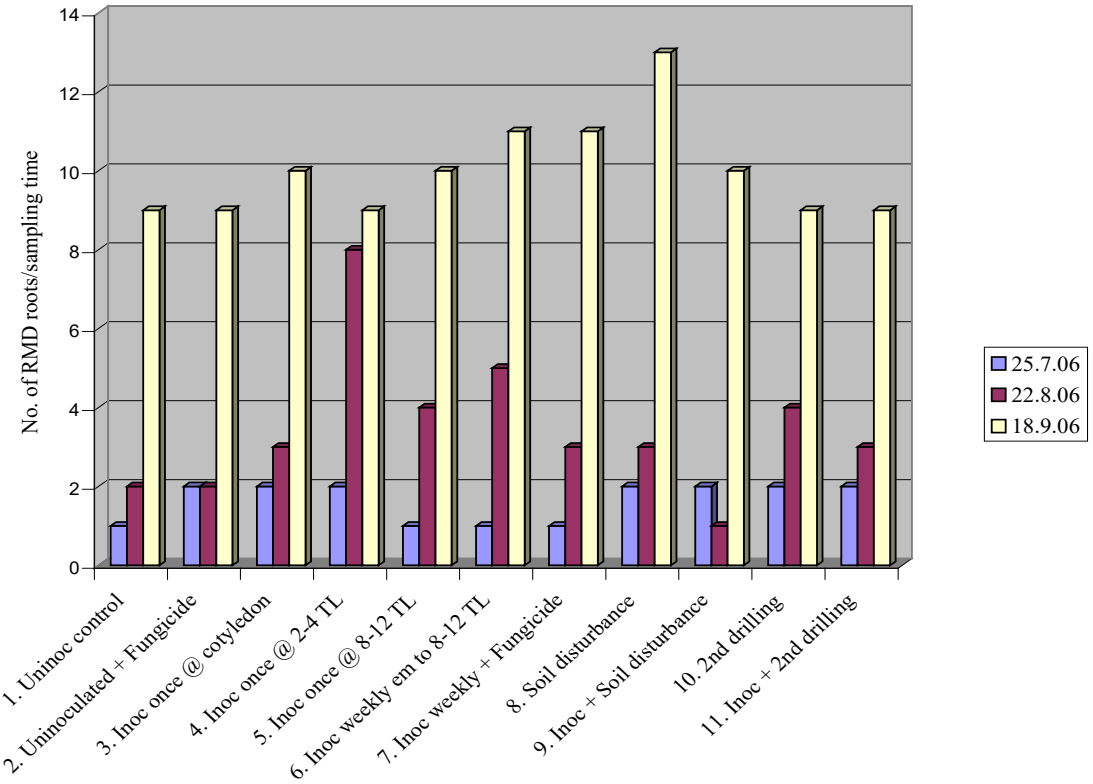
RMD incidence in 2nd Sowing



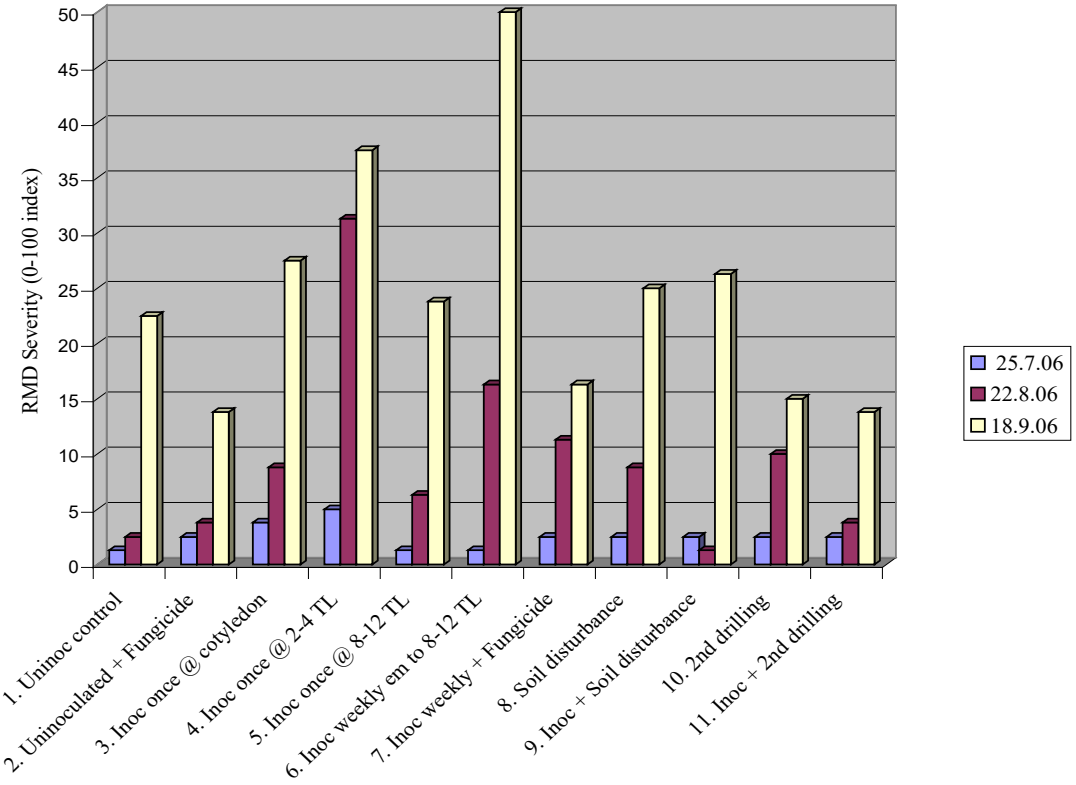
RMD severity in 2nd Sowing



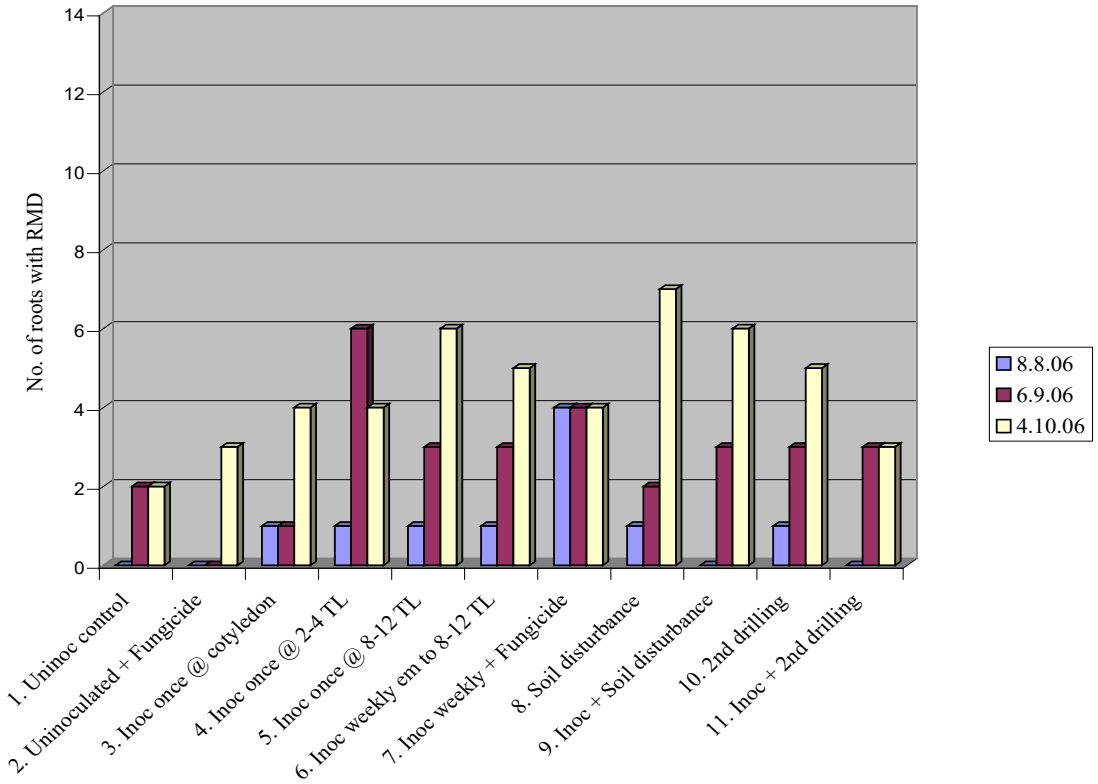
RMD Incidence in 3rd Sowing



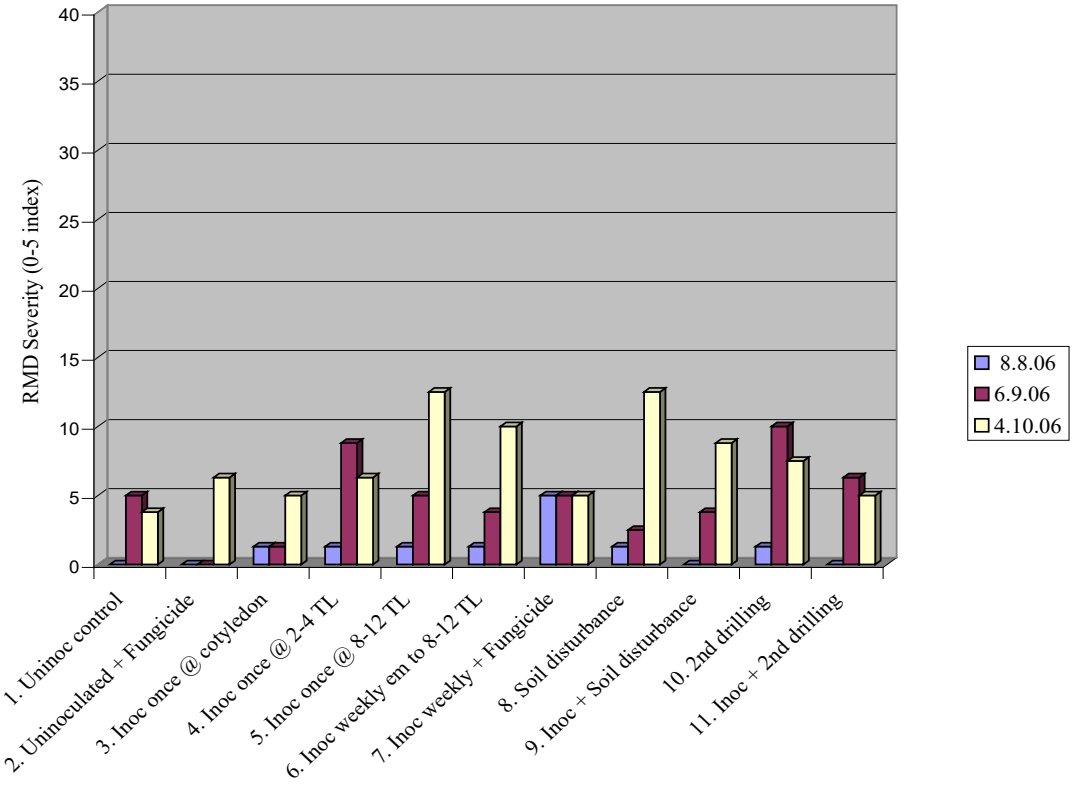
RMD Severity in 3rd Sowing



RMD Incidence in 4th Sowing



RMD Severity in 4th Sowing



1st Sowing

Appendix 4 – Full harvest data sets from the four sowings at STC

Treatment	Plots	% harvested beet with RMD	RMD index (0-100)	Incidence of RMD/no. tagged plants
1. Uninoculated control	8	4.8	1.2	0/0
	22	6.8	3.5	0/0
	31	11.1	5.4	0/0
	36	8.6	3.7	0/0
	Mean	**7.8**	**3.5**	
2. Fungicide treatment	1	0.0	0.0	0/0
	17	10.0	6.0	0/0
	26	4.3	0.9	0/0
	42	10.6	4.0	0/0
	Mean	**6.2**	**2.7**	
3. Inoculated @ cotyledon stage	4	0.6	0.1	1/2
	14	4.0	2.4	0/0
	28	3.0	1.2	0/0
	40	12.3	6.4	0/0
	Mean	**5.0**	**2.5**	
4. Inoculated @ 2-4 TL stage	5	12.2	6.6	2/4
	15	38.0	29.6	7/7
	25	41.8	32.7	8/8
	44	48.1	38.2	7/7
	Mean	**35.0**	**26.8**	
5. Inoculated @ 8-12 TL stage	6	1.0	0.4	0/0
	19	16.4	12.2	0/0
	30	19.1	11.0	0/0
	39	15.4	9.0	0/0
	Mean	**12.8**	**8.2**	
6. Inoculated weekly from emergence	9	23.9	15.9	2/2
	12	31.5	23.8	5/6
	33	67.0	59.4	11/11
	43	37.2	27.7	4/4
	Mean	**39.9**	**31.7**	
7. Inoculated weekly + fungicide every 14 days	7	6.0	1.9	0/0
	21	22.7	15.5	2/2
	27	15.0	10.3	1/1
	41	26.9	17.4	3/3
	Mean	**17.7**	**11.3**	
8. Soil Disturbance	10	3.8	2.1	0/0
	18	6.8	3.8	0/0
	24	11.8	4.5	0/0
	34	12.1	6.2	0/0
	Mean	**8.6**	**4.2**	
9. Soil Disturbance + weekly inoculation	3	8.4	5.2	4/4
	13	21.4	13.9	3/4
	32	40.9	28.2	4/4
	38	34.1	22.6	3/3
	Mean	**26.2**	**17.5**	
10. Second drilled row	11	6.0	2.2	0/0
	16	5.1	2.8	0/0
	23	11.8	5.3	0/0
	37	12.5	7.8	0/0
	Mean	**8.9**	**4.5**	
11. Second drilled row + weekly inoculation	2	13.8	8.3	3/3
	20	31.9	22.8	9/9
	29	55.0	36.8	13/13
	35	40.5	28.4	8/8
	Mean	**35.3**	**24.1**	

2nd Sowing

Appendix 4 – Full harvest data sets from the four sowings at STC

Treatment	Plots	% harvested beet with RMD	RMD index (0-100)	Incidence of RMD/no. tagged plants
1. Uninoculated control	8	2.7	1.3	0/0
	18	8.4	6.2	1/1
	28	14.9	8.0	1/1
	38	4.5	2.5	0/0
	Mean	**7.6**	**4.5**	
2. Fungicide treatment	10	0	0	0/0
	19	0	0	0/0
	23	1.8	1.1	0/0
	36	0	0	0/0
	Mean	**0.45**	**0.3**	
3. Inoculated @ cotyledon stage	2	27.1	14.3	1/1
	14	18.9	11.4	1/1
	29	12.9	10.0	0/0
	34	13.2	8.1	2/2
	Mean	**18.0**	**10.9**	
4. Inoculated @ 2-4 TL stage	7	8.2	5.6	1/1
	17	24.1	20.0	2/5
	32	18.4	11.7	2/2
	42	25.5	17.7	7/7
	Mean	**19.1**	**13.8**	
5. Inoculated @ 8-12 TL stage	1	24.7	11.2	0/0
	13	3.0	0.8	0/1
	33	12.1	7.7	0/0
	37	6.7	4.0	0/0
	Mean	**11.6**	**5.9**	
6. Inoculated weekly from emergence	3	27.1	16.6	1/1
	16	11.6	6.4	1/2
	25	15.3	9.9	1/3
	43	16.9	10.6	3/4
	Mean	**17.7**	**10.9**	
7. Inoculated weekly + fungicide every 14 days	9	2.2	1.5	0/0
	21	2.6	1.8	0/0
	27	0.0	0.0	0/1
	44	1.2	0.7	1/1
	Mean	**1.5**	**1.0**	
8. Soil Disturbance	5	3.8	2.3	0/0
	12	10.4	5.2	0/0
	26	25.6	15.3	1/1
	35	14.3	10.2	0/0
	Mean	**13.5**	**8.3**	
9. Soil Disturbance + weekly inoculation	11	2.8	2.0	0/0
	15	8.7	4.7	1/2
	30	22.8	14.9	3/3
	39	20.7	13.8	3/6
	Mean	**13.8**	**8.9**	
10. Second drilled row	6	17.2	10.9	3/3
	20	16.8	13.3	0/0
	31	13.5	6.5	0/0
	40	2.2	2.0	0/0
	Mean	**12.4**	**8.2**	
11. Second drilled row + weekly inoculation	4	16.0	10.4	2/2
	22	3.8	3.1	0/0
	24	12.8	8.4	0/1
	41	19.4	16.1	5/6
	Mean	**13.0**	**9.5**	

3rd Sowing

Appendix 4 – Full harvest data sets from the four sowings at STC

Treatment	Plots	% harvested beet with RMD	RMD index (0-100)	Incidence of RMD/no. tagged

				plants
1. Uninoculated control	2	7.1	1.8	2/2
	21	6.9	4.7	3/3
	26	3.1	1.4	1/1
	38	4.2	1.9	0/0
	Mean	**5.3**	**2.4**	
2. Fungicide treatment	1	4.5	0.9	0/0
	13	3.9	1.3	0/0
	30	3.7	1.1	0/0
	35	0.9	0.2	0/0
	Mean	**3.3**	**0.9**	
3. Inoculated @ cotyledon stage	7	30.4	19.0	3/3
	15	19.3	14.1	3/3
	28	37.1	24.0	4/4
	43	33.1	20.8	6/6
	Mean	**30.0**	**19.5**	0/0
4. Inoculated @ 2-4 TL stage	4	22.5	10.8	5/7
	14	29.0	18.9	8/9
	29	26.6	17.3	5/5
	41	28.7	20.0	
	Mean	**35.026.7**	**16.8**	
5. Inoculated @ 8-12 TL stage	3	14.5	7.5	2/2
	20	10.9	5.1	1/1
	24	6.8	2.7	0/0
	40	11.8	4.0	3/3
	Mean	**11.0**	**4.8**	
6. Inoculated weekly from emergence	9	39.6	23.8	6/6
	18	36.5	26.5	5/5
	23	46.4	34.1	5/5
	39	24.6	14.3	3/3
	Mean	**36.8**	**24.7**	
7. Inoculated weekly + fungicide every 14 days	10	5.8	3.7	0/0
	16	6.9	5.9	2/2
	33	4.9	2.4	0/0
	42	2.5	2.5	0/0
	Mean	**5.0**	**3.6**	
8. Soil Disturbance	11	8.3	4.6	1/1
	17	15.5	7.5	3/3
	32	5.0	3.2	0/0
	44	5.8	3.5	1/0
	Mean	**8.6**	**4.7**	
9. Soil Disturbance + weekly inoculation	6	5.6	3.2	1/2
	12	5.6	3.5	1/1
	31	10.7	5.4	2/2
	34	6.9	4.4	1/1
	Mean	**7.2**	**4.1**	
10. Second drilled row	5	8.5	2.9	0/1
	19	8.3	4.3	0/0
	25	6.5	2.7	0/0
	37	4.1	2.0	0/0
	Mean	**6.8**	**3.0**	
11. Second drilled row + weekly inoculation	8	16.0	9.4	5/5
	22	18.0	8.7	3/3
	27	5.2	2.6	1/1
	36	3.9	2.1	1/1
	Mean	**10.8**	**5.7**	

Appendix 4 – Full harvest data sets from the four sowings at STC

Treatment	Plots	% harvested beet with RMD	RMD index (0-100)	Incidence of RMD/no. tagged plants
1. Uninoculated control	8	4.1	2.1	1/1
	22	0.8	0.5	0/0
	32	2.3	1.9	1/1
	38	1.5	1.2	1/1
	Mean	**2.2**	**1.4**	
2. Fungicide treatment	10	0.0	0.0	0/0
	17	0.7	0.7	0/0
	26	0.0	0.0	0/0
	41	0.0	0.0	0/0
	Mean	**0.2**	**0.2**	
3. Inoculated @ cotyledon stage	3	6.3	1.8	0/0
	20	3.0	1.6	2/2
	24	1.7	1.0	0/1
	34	0.9	0.2	1/1
	Mean	**3.0**	**1.2**	
4. Inoculated @ 2-4 TL stage	2	5.5	1.7	0/0
	12	0.0	0.0	0/0
	29	0.7	0.3	0/0
	44	0.8	0.3	0/0
	Mean	**1.8**	**0.6**	
5. Inoculated @ 8-12 TL stage	5	3.3	1.4	1/1
	15	6.9	4.5	3/3
	31	1.9	0.8	0/0
	37	0.8	0.2	0/0
	Mean	**3.2**	**1.7**	
6. Inoculated weekly from emergence	6	9.7	5.4	2/2
	19	2.7	1.8	0/0
	27	8.2	4.2	3/3
	36	2.4	2.0	2/2
	Mean	**5.8**	**3.3**	
7. Inoculated weekly + fungicide every 14 days	9	1.2	0.5	0/0
	18	0.8	0.8	0/0
	33	0.0	0.0	0/0
	39	0.8	0.8	0/0
	Mean	**0.7**	**0.5**	
8. Soil Disturbance	4	11.4	5.8	2/2
	13	0.8	0.8	0/0
	28	3.7	2.4	0/0
	43	0.0	0.0	0/0
	Mean	**4.0**	**2.3**	
9. Soil Disturbance + weekly inoculation	17	5.7	4.5	0/0
	21	2.1	1.5	0/0
	25	5.3	2.9	2/2
	35	0.0	0.0	0/0
	Mean	**3.3**	**2.2**	
10. Second drilled row	1	4.4	1.8	0/0
	16	2.3	1.1	1/1
	23	5.0	3.2	1/1
	40	3.1	2.5	4/4
	Mean	**3.7**	**2.1**	
11. Second drilled row + weekly inoculation	11	2.8	1.6	1/1
	14	1.4	1.3	1/1
	30	1.6	1.4	0/0
	42	0	0	0/0
	Mean	**1.4**	**1.1**	