Project Title:	Red Beet: Investigation into the Cause and Distribution of Root Malformation Disorder (RMD).
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The results and conclusions in this report are based on a series of observations and experiments. The conditions under which these observations and experiments were carried out and the results 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 used as the basis for commercial product recommendations.

Authentication

I declare that this 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 .....

Date .....

Principal Investigator
Horticulture Research International
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Report authorised by .....

Date .....

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Practical Section for Growers

Commercial Benefits of the Project

The problem of Root Malformation in Red Beet, known as Root Malformation Disorder (RMD), has been causing crop losses in the region of 25% of harvested roots over the last 4-5 years. The value of these losses have been estimated to be in the region of £1-2 million a year across the industry. The problem has also affected the processors who have experienced increased wastage due to peeling problems with the deformed roots. It has therefore been imperative to get a better understanding of the RMD phenomenon and pinpoint its causes with a view to controlling the problem in future crops.

Background and Objectives

The project was initiated in 1998 when concerns were raised by red beet growers and processors that there was a serious increase in deformed roots in crops. Roots were exhibiting russetting and corking at the crown with clefts and cracks often producing bulbous shaped roots. Laboratory examination of the mature deformed roots at this time could not link any cause, either pathogenic or non-pathogenic, to the deformities. A two-year project was planned to investigate the problem and commenced in April 1999.

First Year (1999-00)

The initial investigations, in the first year of the study, were aimed at understanding the possible causes of RMD. Since it was not known if these would be biological in nature the investigations were undertaken on a broad basis with the long term aim to become more focused as potential causes were eliminated. The objectives were to:

1. Distribute a questionnaire to growers,
2. Monitor plant growth and symptom development in four commercial red beet crops,
3. Perform a pot trial using soils from RMD affected sites.
4. Literature search.

Final Year 2000-01

Work performed in the final year was aimed at focusing on the pathogens pinpointed as the possible cause of RMD. These were *Pythium, Rhizoctonia* and *Aphanomyces*, which were implicated, in the first year of the study as being involved singly or as a disease complex in the RMD phenomena.

The objectives of the second year were:

1. Perform three field trials using seed and drench treatments to eliminate the suspect pathogens from the emerging seedlings thereby implicating their involvement in initiating RMD.
2. Monitor two commercial red beet crops to assess pathogen presence in first few weeks following emergence.
3. Conduct a field trial using *Rhizoctonia* isolates (collected from RMD affected red beet in the first year of the study) that inoculated into field soils around growing red beet seedlings. The objective was to reproduce RMD symptoms in the red beet.

Summary of Results and Conclusions

First Year (1999-00)

The results from the first year of the project showed that:

1. Increased rainfall during the earlier part of the season, in recent years, had significantly contributed to the occurrence of RMD.
2. The problem had not just arisen in the last 2 years but had been building steadily over a prolonged period with increasing numbers of roots being affected each year.
3. Fungicide seed treatments currently used on red beet, were not having any effect on the occurrence of RMD. This was particularly relevant to the seed treatment – Tachigaren, widely used for the control of *Aphanomyces cochlioides*, which causes 'black leg' and damping-off in red beet seedlings. There were no consistent reports from growers that the use of Tachigaren treated seed was reducing the occurrence of RMD.
4. Different red beet cultivars were equally affected by RMD with no clear differences in occurrence or severity of deformity.
5. The root malformation was being initiated at the seedling stage by factors which were causing small areas of damage to plant stems. If the seedlings survived the early damage to stems then they appeared to develop scar tissue, which later affected the shape of the developing root.
6. Soil-borne plant pathogenic fungi – *Pythium* and *Rhizoctonia,* were regularly associated with minute areas of damage found on the crown and stems of seedlings. Also *Aphanomyces cochlioides* was observed and isolated from seedlings demonstrating blackening and pinching of the stems. Nutritional factors and plant viruses were eliminated as being involved.

7. A literature review complemented the view that *Pythium, Rhizoctonia* and *Aphanomyces* could be associated individually or together in a disease complex as the initiators of the RMD phenomenon.

Final Year 2000-01

1. The field trials clearly implicated both *Pythium* and *Rhizoctonia* as the causes of RMD.
2. The organisms appear to be operating together or separately causing small patches of damage to stems at the seedling stage. The seedlings may die at the seedling stage but if they survive they mature to produce scarring around the tissue damaged earlier. The scar tissue restricts root development causing deformity as the root expands.
3. The deformity is initiated in the first 10 weeks following drilling. After this *Pythium* was not detected on the roots. However *Rhizoctonia* can still be found at this stage and is associated with further russetting of the crown tissue in older roots in some crops.
4. *Aphanomyces* did not appear to be involved in the cause of RMD but was associated with damping-off and black-leg symptoms in non-Tachigaren treated crops.
5. *Rhizoctonia* isolates collected from affected crops in the first year were successfully shown to cause root malformation when added to field soil in which red beet was grown.
6. The wetter weather seen in the Spring season of recent years appears to have exacerbated the build up of *Pythium* and *Rhizoctonia* inoculum in red beet soils.
7. Differences in topography and soil conditions affect the distribution of *Pythium* and *Rhizoctonia* in RMD affected crops. *Pythium* primarily occurs in wet and less well-drained sites. *Rhizoctonia* also occurs in wet and poorly drained soils but is also found in drier and better-drained locations.
8. Improvements in seedling vigour appear to reduce the occurrence of RMD. Later sown crops, which avoided the wetter and cooler weather of Spring and produce a more vigorous seedling, appear to be generally less affected by RMD.

Action Points for Growers

- The causes of root malformation in red beet are due to infection at the seedling stage by the fungal pathogens *Pythium* (*ultimum*) and *Rhizoctonia* (*solani*).

- The occurrence of these organisms and therefore deformity in red beet is exacerbated by the increasingly wet spring weather as seen in recent years.

- Currently no fungicides are registered for use against *Pythium* or *Rhizoctonia* in red beet. There is also some difficulty in exploring the use of fungicides to control RMD, at this present time, because the two organisms involved are unrelated and would require the use of two different fungicides. The fungicide SL567A (Metalaxyl-M) showed excellent reduction of RMD at one site where *Pythium ultimum* was the major cause of deformity. However at a site where *Rhizoctonia* was the primary cause, and to a lesser extent *Pythium*, SL567A was less effective.

- If the use of fungicides to control *Pythium* and *Rhizoctonia* is required by the industry then additional work would need to be carried out to secure on or off-label approval for any such fungicides, applied as pre or post emergent sprays, on red beet crops.

- It is apparent that improvements in seedling vigour help to reduce the damage caused by these two pathogens and therefore reduces subsequent malformation. The use of Biomex SA (biological soil conditioner from Omex Agriculture) as an experimental seed treatment increased seedling vigour and reduced the occurrence of RMD at two sites. It is therefore possible that similar 'biological' vigour enhancing products could help seedlings to grow through the 'risk' period from emergence to 8 weeks post-drilling. The availability, timing and application of such 'biological' products should be explored further.

- Later drilled crops (June) are generally less affected.

- It is important to avoid poorly drained, compacted or excessively wet land for red beet cropping.

- The effect of crop rotation on the occurrence of RMD has not been explored within the remit of this project but it is likely that crops included in a typical rotation with red beet affect the occurrence of *Pythium* and *Rhizoctonia*. This topic would require further study to determine if rotations could be altered to reduce RMD. It is widely known, for example, that different *Rhizoctonia* strains occur on different crop types and that the *Rhizoctonia* recorded on red beet may or may not be the same strain that attacks potatoes.

Anticipated Practical and Financial Benefits

The project has delivered some clear indications to growers as to the causes of root malformation and has outlined the environmental parameters that affect the occurrence of RMD in red beet crops. This new information will now allow growers to plan future cropping and understand the risks.

Science Section

Introduction to Science Section

The second year of this study is divided into three sections. Firstly, replicated trials (seed and drench treatments) conducted on grower sites; monitoring of two red beet crops from red beet affected areas and finally, a replicated *Rhizoctonia*/red beet incorporation trial conducted at HRI – Stockbridge house using *Rhizoctonia* inoculum collected from RMD affected crops in the first year of the project.

1. Replicated Fungicide Trials

1.1 Introduction

Three field trials were planned using a range of experimental seed and drench treatments aimed at targeting *Pythium*, *Rhizoctonia* or *Aphanomyces* and, by a process of elimination, demonstrate their individual or combined involvement in the development of RMD. The trials were operated at three commercial sites in Lincolnshire and Yorkshire and included six experimental seed treatments onto which were superimposed four experimental fungicide drenches and one biological growth enhancer (Biomex SA) all of which were applied 5-6 weeks post drilling. The five drench treatments were compared to a water control treatment. The biologically active product Biomex SA contains a living fungal organism (*Trichoderma* sp.) and was included in the treatments because of its reported affects on the establishment of seedlings. The product is used as a pre-plant soil conditioner to improve seedling vigour and therefore could help the emerging roots and shoots produce stronger more vigorous growth and escape attack from the pathogens that are initiating RMD.

1.2 Materials and Methods

Crop and Cultivar

Red Beet cv Crimson Globe (Thiram soaked)

The Trials - Design and Sites

The trial consisted of six different seed treatments onto which were superimposed 6 different spray treatments. The trial was repeated at three different farm sites in the middle of commercial red beet crops. The seed treatments were drilled using commercial drill equipment in discrete blocks. The size and arrangement of these blocks varied slightly between sites depending on the equipment being used. Onto the blocks of drilled treated seed were superimposed 6 spray treatments that were applied post-emergence. These 6 treatments were replicated 3 times forming a randomised block of spray treatments on top of each seed treatment. Plots were approximately 4m x 1.8m comprising of 3 rows of plants at Site 1 and a complete raised bed (6 rows of plants) at Sites 2 and 3. Trial plans showing the trial design at the three sites are shown in Appendix I.

Site 1 at Spilsby in Lincolnshire was located on an organic soil type
Site 2 at Westwoodside in South Yorkshire was located on a silty loam soil type.
Site 3 at West Butterwick in Lincolnshire was on a warp soil type.

The Treatments

	Seed Treatments	**Target Pathogen**
A	Untreated Control – Thiram (Thiram 600g/l) soaked (standard)	Control
B	Thiram (Thiram 600g/l) soaked and SL567A (metalaxyl–M 2ml product/Kg seed) coated	*Pythium*
C	Thiram (Thiram 600g/l) soaked and Tachigaren (hymexazol – 21g product/Kg seed) coated	*Aphanomyces*
D	Thiram (Thiram 600g/l) soaked and Monceren Flowable (pencycuron - 16ml product/Kg seed) coated	*Rhizoctonia*
E	Scarified (acid treated) and Biomex SA (Trichoderma from Omex - 15ml/Kg seed) soaked	Improved Seedling Vigour (Non-target specific)
F	Thiram (Thiram 600g/l) soaked and Monceren Flowable (pencycuron - 16ml product/Kg seed) and SL567A (metalaxyl–M - 2ml product/Kg seed) coated	*Rhizoctonia* and *Pythium*

	Spray Treatments	Target Pathogen
1	Untreated Control	Control
2	SL567A (Metalaxyl–M) 1.3 l product/1000 l water/ha	*Pythium*
3	Tachigaren (Hymexazol) 2 Kg product/1000l water/ha.	*Aphanomyces*
4	Basilex (Toclofos-methyl) 10 Kg product/1000l water/ha	*Rhizoctonia*
5	Amistar (Azoxystrobin) 6.0 l product 1000l water/ha.	Broad-Spectrum Range of Pathogens
6	Biomex SA (Trichoderma from Omex) 124 ml product 1000l water/ha.	Improved Seedling Vigour

Application of Spray Treatments

Spray treatments were applied on one occasion at 5-6 weeks post drilling when plants were at the 3-4 true leaf stage. Fungicides were applied using a lance attachment on an Oxford Precision Sprayer modified to operate with compressed air at a pressure of 2 bars.

Treatments 3 (Tachigaren) and 4 (Basilex) were rinsed off of the foliage following application to minimise the risk of leaf scorching. The rinsing was performed by spraying water at 1000l/ha. in a 1-2 hour period following fungicide application. This precaution was taken due to the known risks of Basilex causing phytotoxic symptoms under hot field conditions. Also the application of Tachigaren, as a drench, is not approved and there were therefore some risks of possible leaf scorching when applied to foliage.

Assessments

The assessment of the severity of root malformation was performed on two occasions. The chart used for assessing RMD severity is included in Appendix II. Assessments were performed on 50 roots randomly harvested from each plot. The first assessment was performed mid-season and the second at harvest in the autumn.

Crop Diary

	Site 1 – Spilsby	Site 2 – Westwoodside	Site 3 – West Butterwick
Drill Date	18 May 00	8 June 00	19 May 00
Spray Date	26 June 00	17 July 00	27 June 00
First Assessment	9 August 00	15 August 00	15 August 00
Final Harvest Assessment	25 September 00	3 October 00	26 September

Statistical Analysis

Raw data was square root transformed. A statistical analysis of variance was performed on transformed data using a Genstat 5 programme. Results are displayed in Tables 1-6 in Appendix III. Within the tables of results are comments on the significance of data. The notation of significance in the tables is based on the following: -

NS = Result not significant,

* = Significant result (P at 5 %),

** = Highly significant result (P at 1%),

*** = Very highly significant result (P at 0.1%).

1.3 Results

The results for mean RMD severity across the three trial sites are displayed on the following six pages as graphs (histograms). Two graphs are given for each of the trial sites showing results for the mid-season and final harvest assessments respectively.

It should be noticed that the scale of RMD severity shown on the left-hand axis (Y-axis) of the graphs varies for each trial site depending on the level of severity at the particular assessment. It is therefore important, if performing a visual comparison between sites, to take these differences into consideration.

The Tables of statistically analysed results (square root transformed) are displayed in Appendix III.

Figure 1: Site 1 - Severity of Root Malformation Disorder (RMD) at First Harvest (9 August 00)

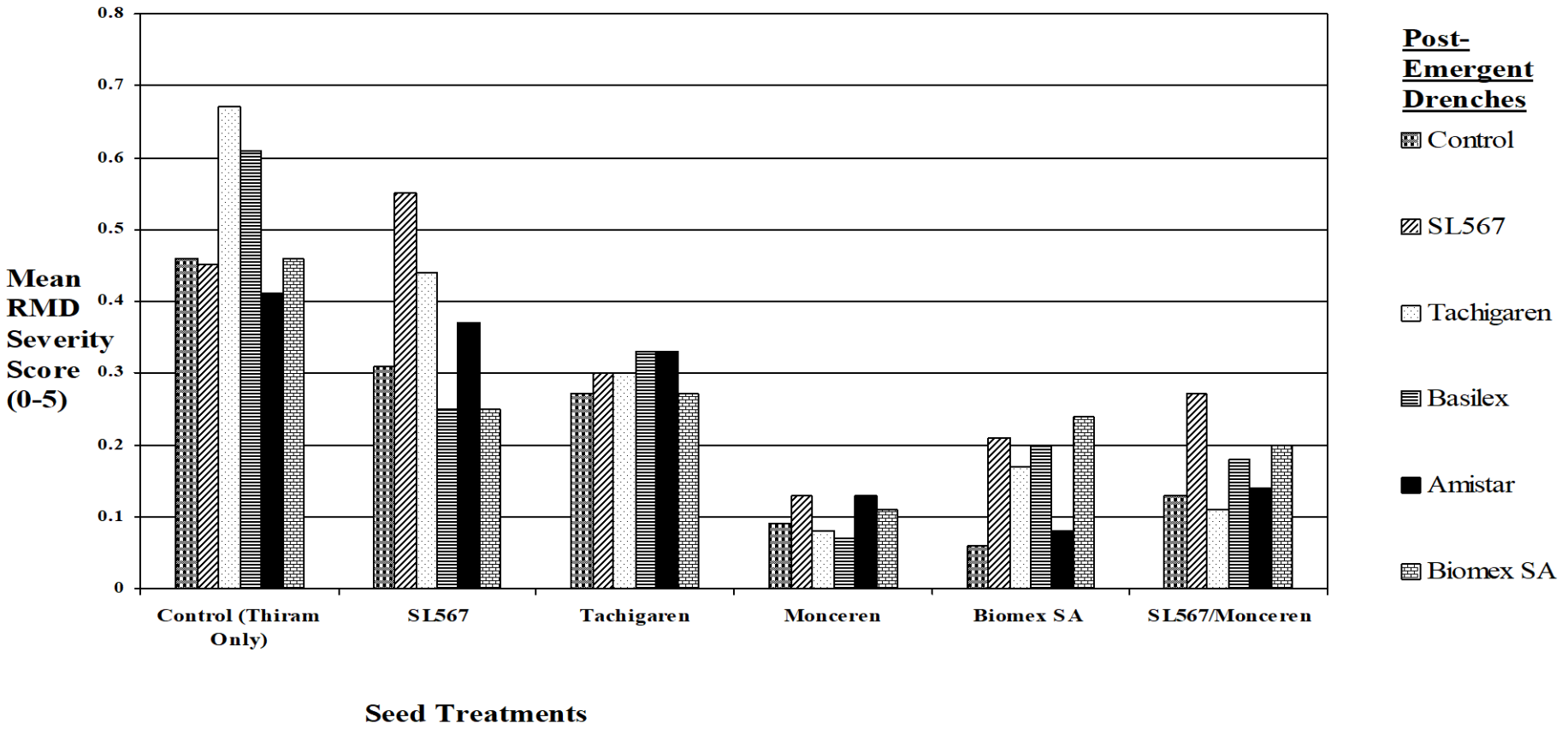


Figure 2: Site 1 - Severity of Root Malformation Disorder (RMD) at Final Harvest (25 September 00)

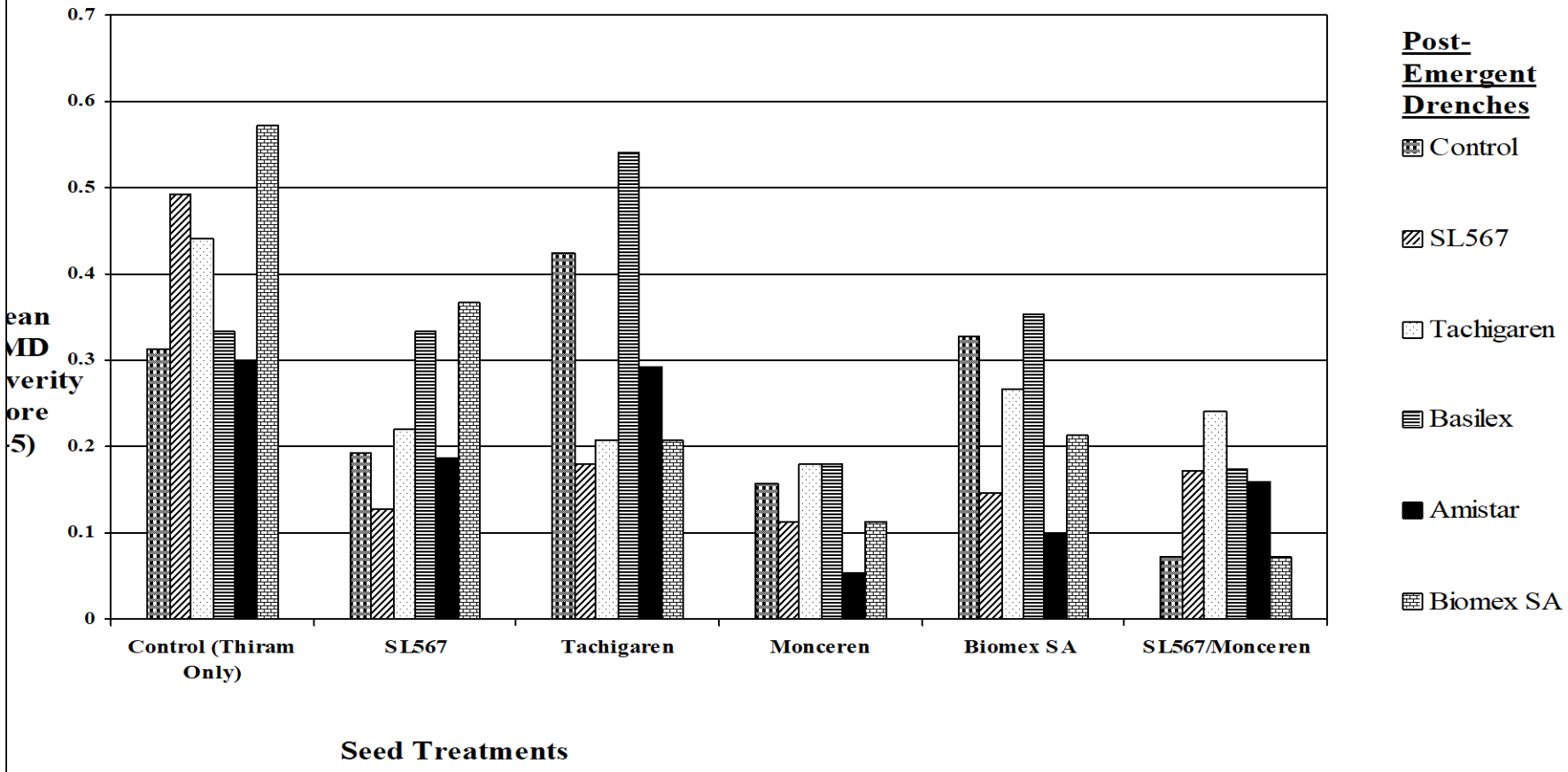


Figure 3: Site 2 - Severity of Root Malformation Disorder (RMD) at First Harvest (15 August 00)

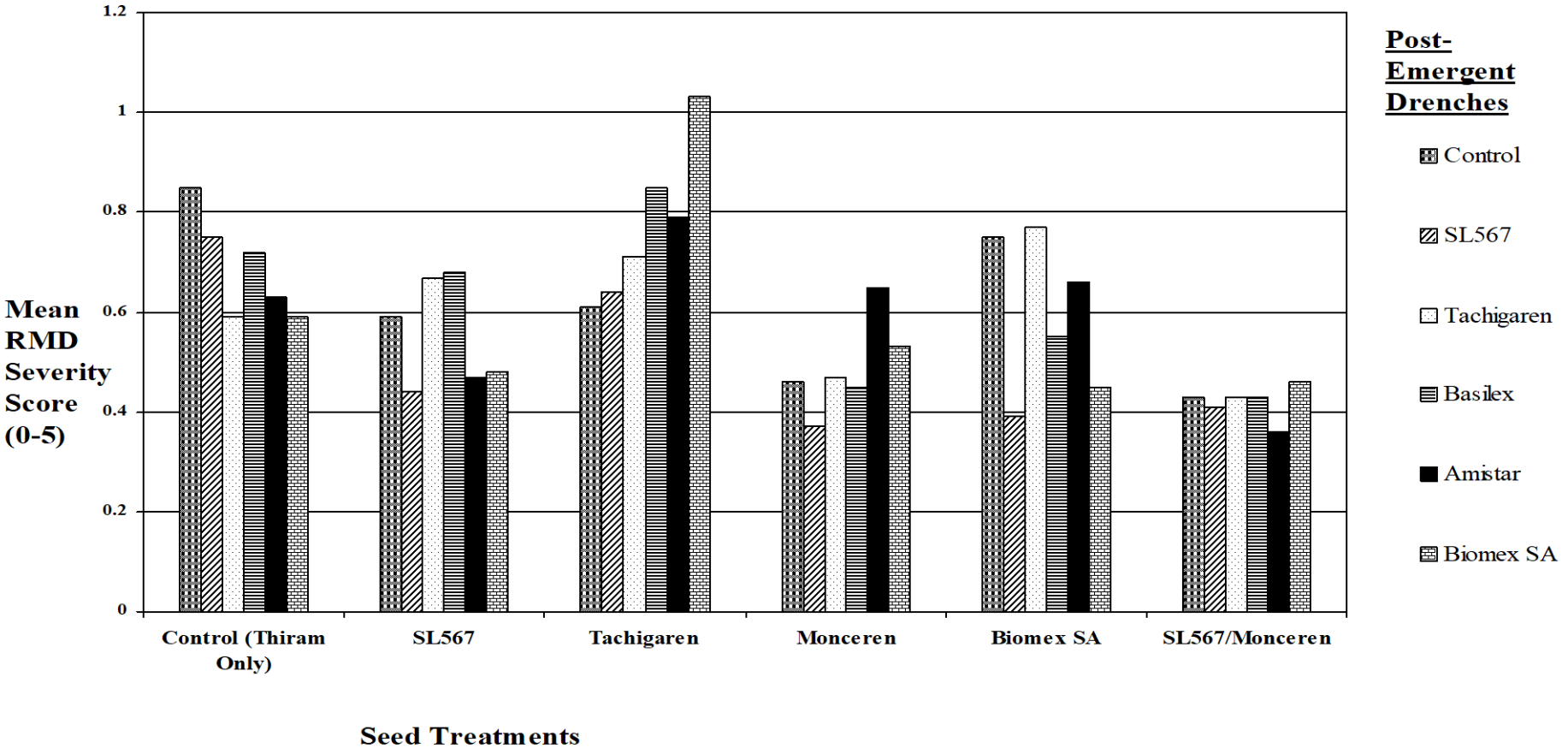


Figure 4: Site 2 - Severity of Root Malformation Disorder (RMD) at Final Harvest (3 October 00)

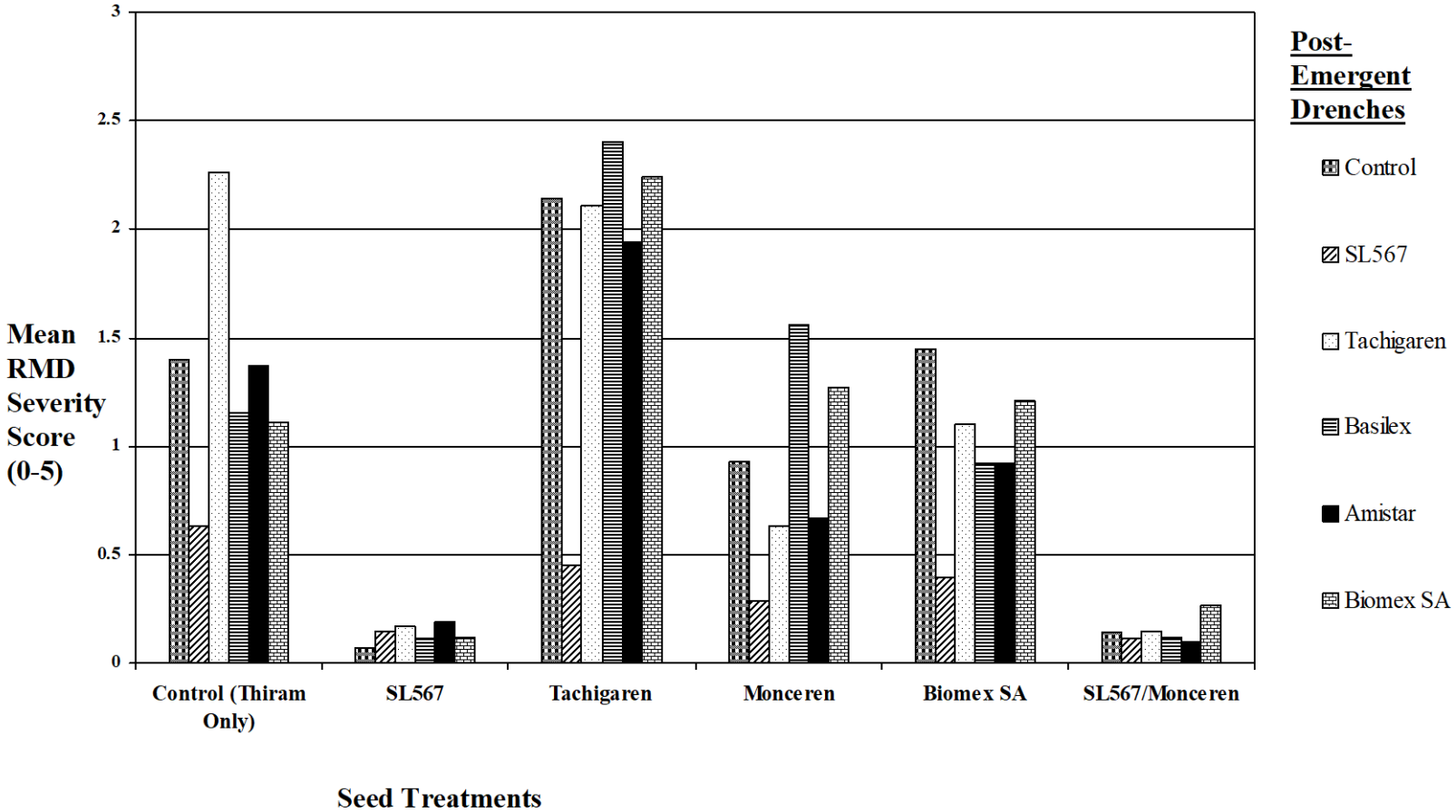


Figure 5: Site 3 - Severity of Root Malformation Disorder (RMD) at First Harvest (15 August 00)

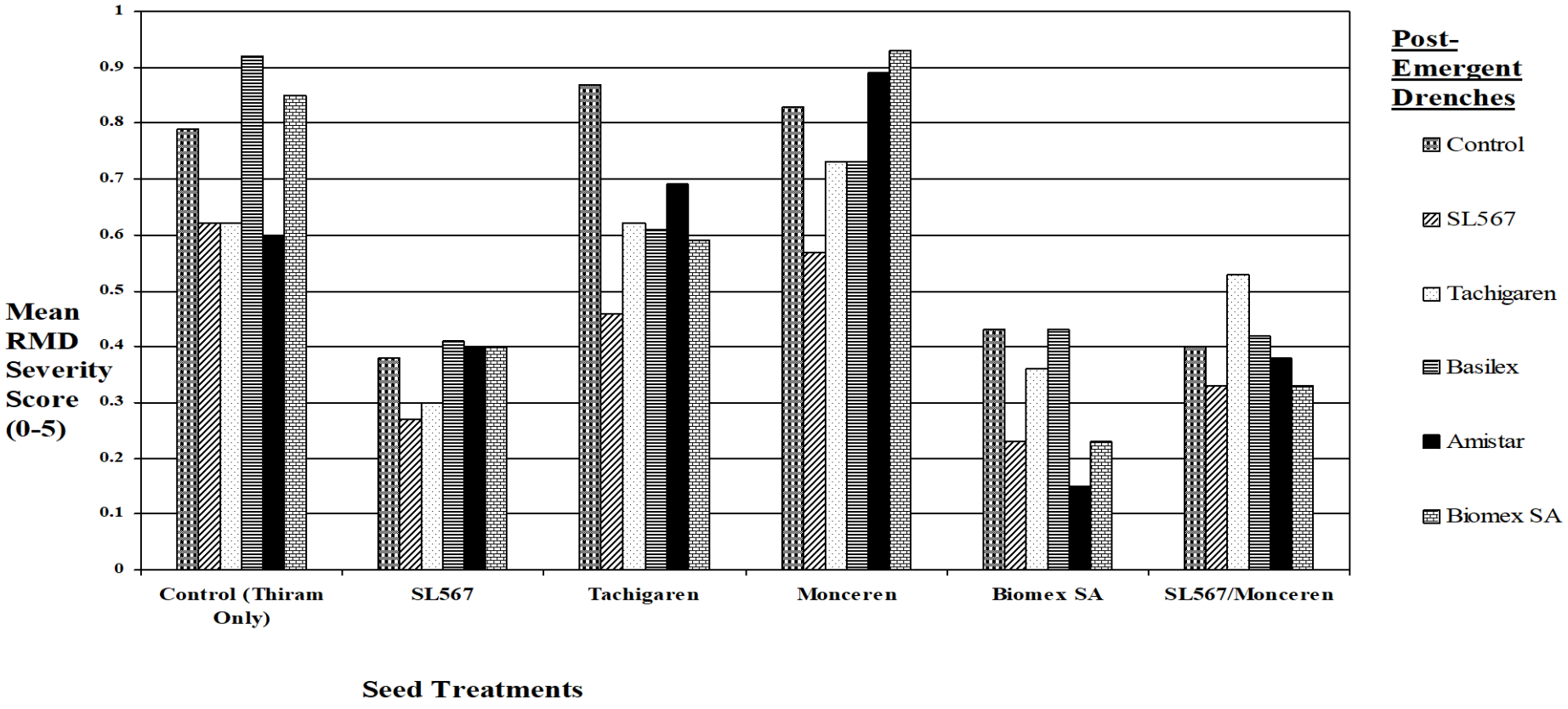
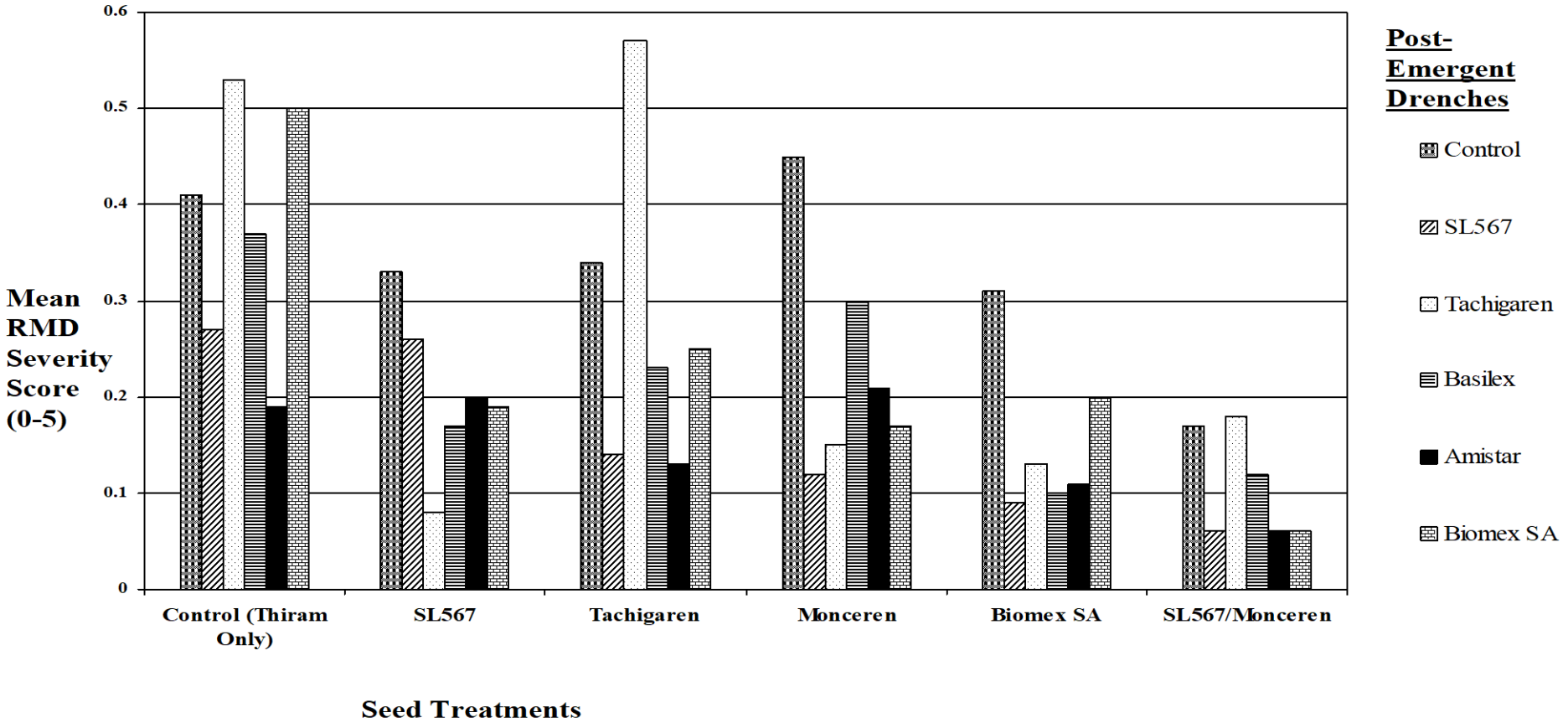


Figure 6: Site 3 - Severity of Root Malformation Disorder (RMD) at Final Harvest (26 September 00)



1.4 Discussion of Results

Root malformation was recorded in the red beet at all three trial sites. However, based on observations of affected crops in 1999, the severity of malformation was less than expected during the 2000 season. It was noted that RMD severity was generally less than expected not only at the trial sites but also across the eastern counties where a number of key growers had observed less deformity than in the previous year. Amongst the trial sites the best response to the treatments, both seed and drench, was seen in the most severely affected site. This was Trial Site 2–Westwoodside, with severity levels reaching as high as 2.0-2.5 (see Appendix II for severity index) in the untreated control treatment. Sites 1-Spilsby and Site 3-West Butterwick were less affected with the highest RMD severity scores being below 1.0.

At the first assessments in August the plants at each site were in the early stages of stem expansion and the production of a normal red beet shape. At this mid-season assessment plants that were assessed as being affected by the early stages or low levels of RMD often had recovered to produce normal roots by the final (harvest) assessment. This was particularly the case at Site 3 – West Butterwick, where the levels of RMD mid-season were higher and with more differences between treatments than at the final assessment.

The treatment means, as displayed in Figures 1-6, show that the overriding effect in each of the trial sites is that of the seed treatment with differences between drenches often a backdrop to the effects of the seed treatments. However there is considerable variability in the data, some of which is related to treatment effects but also to the patchy nature of the RMD phenomenon within the field. Also the design of the trial with seed treatments in large (unreplicated) blocks, due to the restraints of sowing machinery, precludes any comparison of the seed treatments to estimate the levels of significance between the seed treatment results. In this instance the use of the Variance Ratio (for seed treatments) can be used to estimate if there is any extra variability in the data which would indicate that there was a seed treatment affect. If the calculated Variance Ratio is less than the tabulated critical value (as determined by F distribution tables at 5% at 5 and 12 degrees of freedom) then there is no evidence of differences between the seed treatments. If, however, the Variance Ratio is greater than the critical value then there are significant differences and the extra variability in the results can be explained by a seed treatment affect.

Using the Least Significance Difference to compare individual results also helps to tease out treatment effects from the results even when there is too much variability in the results to be significant. The Tables of results showing the analysed data with Variance Ratio's and Least Significant Differences (LSD) are shown in Appendix III.

Site 1 – Spilsby

The trial at this site was situated in a relatively dry and well-drained area of the field. The land had been cropped with red beet in the previous year and RMD had been recorded in the site.

The results for Site 1 – first harvest (Figure 1) show significant differences for seed treatments but drench treatments are not significant (P=5%). To examine the effect of seed treatment alone, without the influence of the post-emergent drenches, the results in the first column of the Figure 1 (Table 1) have to be examined. These results show that treatments D (Monceren Flowable), E (Biomex SA) and F (Monceren Flowable/Sl567A) all produced significantly lower levels of RMD than the untreated control. Biomex SA seed treatment had the lowest levels of RMD at this assessment. A Basilex drench superimposed on the Monceren Flowable seed treatment was the best performing drench treatment at this assessment.

The results for the seed treatments at the final harvest (Figure 2, Table 2) are not significant but some individual comparisons using the LSD for this assessment show that treatment F (Monceren Flowable/SL567A) produced the lowest severity of RMD with Treatment D (Monceren Flowable), a close second. The results for drench treatments at this final assessment were significant (P=5%). SL567A as a drench was also effective across seed treatments B (SL567A), D (Monceren Flowable) and F (Monceren Flowable/SL567A). Drench treatment 5 (Amistar) in combination with Monceren Flowable seed treatment reduced RMD severity and drench treatment 6 (Biomex SA) when used with Monceren Flowable/SL567A seed treatment both reduced RMD to the greatest extent.

The results from Site 1 indicate that the fungicide Monceren Flowable is effective in reducing RMD at this site. The seed treatment containing both Monceren and SL567A (Treatment 6) was also effective but since SL567A alone did not significantly reduce RMD severity it can be deduced that it is the Monceren Flowable content of this treatment that is being effective against the development of RMD at this assessment. Monceren is effective against the fungal organism *Rhizoctonia* and it is therefore reasonable to assume that *Rhizoctonia* is actively involved in the RMD phenomenon at this site. The crop growth enhancer was also effective in reducing RMD severity at this assessment by improving seedling vigour in the early stages of root formation and thereby allowing the seedling to combat invasion by the fungal pathogen.

Similar results were seen at the final harvest for this site with *Rhizoctonia* implicated as involved in the development of RMD symptoms. However the improved activity of SL567A as a drench treatment at this assessment also implicates the involvement of *Pythium* in the RMD phenomenon. Again the combined seed treatment of Monceren Flowable and SL567A provided good control of RMD which, following the positive result gained from the use of a SL567A drench, could implicate both *Rhizoctonia* and to a lesser extent *Pythium* in the development of RMD at this site.

Site 2 - Westwoodside

This trial site was located in a field to which the rest was planted to red beet. The site was selected because it was particularly wet land and expected to have RMD problems. The site began the season being waterlogged due to persistent wet weather with sowing being delayed until early June.

The results for the first harvest (Figure 3, Table 3) at Site 2, show no significant differences between the seed and drench treatments. However the final harvest for this site shows some striking and significant differences between seed and drench treatments. The results for seed treatments alone, without the influence of drenches (Figure 4, Table 4) show significantly less root malformation in seed treatments B (SL567A) and F (Monceren Flowable/SL 567A). Throughout the trial SL567A used as a drench also controlled the development of root malformation across all the seed treatments. SL567A showed significant and improved control across all seed treatments with the exception of treatments B (SL567A) and F (Monceren Flowable/SL 567A) where the controlling effect of the seed treatments reduced the

differences between the drenches producing no significant differences between drenches on these treatments.

The results for the final harvest at this site show the most significant effects of seed treatment amongst the three trial sites. The good control of RMD demonstrated by SL567A indicates that *Pythium*, which is being controlled by this fungicide, is implicated in the development of RMD. SL567A is the only drench treatment, which consistently shows a reduction in RMD severity despite the seed treatment onto which it was superimposed. There were very pronounced differences in the appearance of plots in this trial with SL567A treated plots having healthier and more prominent foliage than adjacent plots. The wet nature of the land at this site during sowing and in the first few weeks following drilling would have actively encouraged the growth and infestation of roots with *Pythium* which thrives in wet soils.

Site 3 – West Butterwick

The trial was situated in a site to which the rest of the field was sown to red beet. The site did not have any immediate history of RMD but was on land expected to develop the phenomenon. The site was relatively well drained but following periods of heavy rain became quickly sodden.

The results for Site 3 – first harvest (Figure 5, Table 5) show significant differences for seed and drench treatments (P=5%). The results for seed treatments alone, without the influence of drenches show that treatment B (SL 567A), E (Biomex SA) and F (Monceren Flowable/SL567A) all reduced RMD severity. Drench treatments 5 (Amistar) and 6 (Biomex SA) when superimposed on the Biomex SA seed treatment produced the lowest levels of RMD severity.

The results for the seed and drench treatments for Site 3 at the final harvest (Figure 6, Table 6) are not significant. There are some small difference in individual treatments at this harvest but the levels of RMD are so low which coupled with the variability of the results make such comparisons unrealistic.

SL567A again showed better control of RMD severity at this site which again implicates *Pythium* infection as a causal agent in root malformation.

In each of the trials the fungicide Tachigaren did not show significant control of root malformation. Tachigaren was included in the trial because it targets *Aphanomyces* and would therefore by its control implicate its involvement in RMD. In some of the results Tachigaren did appear to have some effect, particularly as a drench treatment on top of a seed treatment that was effective. However these results were not consistently shown across the sites and at different harvests and in each case were not significant. While the activity of *Aphanomyces* cannot be entirely ruled out it did not appear to be a cause of RMD at the sites examined.

2. Crop Monitoring

2.1 Introduction

Following consultation with red beet growers, two sites, which were identified as being at risk from developing RMD, were selected for crop monitoring. One site in Yorkshire and the other in North Lincolnshire. Each of the sites were on a farms that had a history of RMD in red beet in the last 2-3 years.

The results of crop monitoring performed in the first year of this study indicated that the symptoms of RMD were initiated in the first 10 weeks after emergence. Sampling in this year's work was therefore concentrated in this early period following emergence. Both sites were visited at regular intervals from emergence onwards. Initially beet plants were sampled randomly from the crop but as sampling progressed, certain 'hot spots' became identified in the various crops. These usually corresponded to compacted or poorly drained areas of soil within the sites. Samples consisted of 10-15 plants sampled from up to 20 different locations within the site. The samples were returned to the laboratory where detailed microscopic examination and isolations were performed on the seedlings to detect the presence of any plant pathogenic organisms.

Isolations were performed using a range of general and selective culture media. These included *Pythium* Selective Agar (Jeffers & Martin, 1986), Water Agar, *Aphanomyces* Selective Agar (Pfender et al, 1984) and Novobiocin amended Potato Dextrose Agar.

2.2 Results of Crop Monitoring

The table below gives the drilling and sample dates for each of the two monitored sites:

	Site 1 – Lincs	Site 2 – Yorks
Variety and Seed Treatment:	Crimson Globe (Thiram soaked)	Pablo (Thiram and Tachigaren Treated)
Crop Drill Dates -	8 May 00	8 June 00
Dates Samples Collected:	24 May 00	20 June 00
	6 June 00	27 June 00
	16 June 00	4 July 00
	27 June 00	13 July 00
	4 July 00	17 July 00
	13 July 00	28 July 00

Site 1 - Lincs

The samples collected in the first two site visits were largely healthy seedlings but included 3-4 seedlings per sample with microscopic areas of damage to the side of stems. Examination of these seedlings consistently detected either *Pythium* or *Rhizoctonia* in association with these affected areas. Other seedlings showed pinching and a 'blackleg' type symptoms to the stem. These seedlings appeared more seriously affected than those with small patches of damage. *Pythium* and *Aphanomyces* were found in association with these more seriously affected seedlings.

As the sampling progressed the seedlings, which earlier displayed small patches of necrosis, either became 'wirestemmed' and died or appeared to overcome the earlier damage and grow-on but with areas of scar tissue which resulted in abnormal stem and root shape.

The more seriously affected seedlings from which *Pythium* and *Aphanomyces* was isolated disappeared from the field by the third and fourth sampling. It was apparent that they had mostly died in what had been a blackleg or damping-off stage of *Pythium* or *Aphanomyces* infection.

By the fourth site visit affected roots were producing distinct areas of deformity from which it was easy to see how they would mature to become typical RMD affected red beet. Microscope examination and isolations performed on these affected areas of the roots detected *Pythium* or *Rhizoctonia* either together or separately on these roots. Of the *Pythium* isolates occurring on the red beet, six of the commonly occurring colony types were forwarded to Dr Tim Pettitt, HRI, Wellesbourne for species identification. Four of the six were identified as *Pythium ultimum*. The other two could not be clearly identified. The *Pythium* isolates identified as *Pythium ultimum* comprised the greater number of *Pythium* species being detected in RMD affected beets.

At the sixth site visit it became increasingly difficult to associate any pathogens with RMD symptoms as roots became hardened as they matured. Sampling was therefore finalised at the seventh visit on 13 July.

Site 2 – Yorks

Samples collected in the first two site visits contained 4-5 seedlings per sample which were displaying severe pinching under the crown area of the plants. A number of roots also displayed a 'blackleg' type symptom as seen in plants from Site 1. Isolations performed on these plants consistently detected the plant pathogen *Pythium*. The *Pythium* isolates were subsequently identified as *Pythium ultimum*. Subsequent isolations performed on the samples from this site consistently detected *Pythium ultimum* in association with any form of stem damage whether it was a small area of pinching or depressions on the sides of developing stems.

Rhizoctonia was detected on plants in 2-3 samples at each of the final two site visits. In each case *Rhizoctonia* was found on the plants in association with russetting and scarring on the crown area of developing plants.

2.3 Discussion of Crop Monitoring Results

Beet from both of the sites visited grew on to develop moderate to severe levels of root malformation. In each case the RMD symptoms were more severe in compacted areas of the crop especially around gateways and areas where machinery had travelled more often and in depressed and less well-drained areas of the field. However these two sites also had a fairly even distribution of RMD affected plants throughout the whole crop irrespective of topography.

It appeared that both *Rhizoctonia*, identified as *Rhizoctonia solani*, and *Pythium ultimum* are involved as main causal agents in the RMD phenomenon. They were consistently isolated from roots where they were causing damage to developing stems. This damaged area then caused scar tissue which affected the normal development of red beet root shape. Their involvement appears to vary at different sites. *Rhizoctonia* and *Pythium* were present in similar levels both on the same plants and on different plants at Site 1 but at Site 2 *Pythium* was the most dominant organism.

Aphanomyces was also found in association with *Pythium* at Site 1 and could also be contributing to the development of root deformity. However it was noticed that the roots on which *Aphanomyces* was found were more classically 'blackleg' affected and often did not survive the seedling 'blight' stage of the disease. *Aphanomyces* was not found on any seedlings from Site 2 and this was attributed to the Tachigaren seed treatment used on this crop.

At each site it was quite possible to walk the crop and lift up plants which showed the typical early stages of root deformity. In each case these plants were less thrifty and had purple coloured foliage indicating nutritional stress due to loss of root function. By the 10th week following emergence many of these unthrifty plants had recovered and had begun to grow normally. These plants subsequently showed typical RMD symptoms to varying degrees at maturity.

3. Rhizoctonia Incorporation Trial

3.1 Introduction

Following the first year of the study it was suggested that a small trial using *Rhizoctonia* isolates collected from RMD affected red beet crops, during the crop-monitoring phase of the work, could be used to inoculate red beet seedlings to recreate the RMD symptoms as seen in mature red beet. This trial was therefore conducted at Stockbridge House in a field site using a mixture of isolates of *Rhizoctonia* taken from RMD affected red beet seedlings in 1999.

There are two main inherent difficulties in amending soil with plant pathogens to cause infection in plants. Firstly insuring that the level of infection introduced is high enough to cause infection and secondly that the infection causes the symptoms required without killing the plants. It was therefore decided that two levels of *Rhizoctonia* inoculum would be used in the trial. These levels could not be determined by any prior testing owing to the time constraints of starting the trial at the correct time of year. A guide to inoculum levels was therefore obtained from previously documented trials in the USA (Olaya & Abawi, 1994; Ruppel, 1985).

3.2 Materials and Methods

Crop and Cultivar

Red Beet cv Crimson Globe (Thiram soaked).

The Trial - Design

The trial consisted of three treatments – two concentrations of *Rhizoctonia* inoculum which were amended to surface soil and compared to an untreated control. The treatments were replicated three times and arranged in a complete randomised block.

Inoculum Preparation

The *Rhizoctonia* inoculum used in this trial was obtained from the surface of RMD affected red beet plants during the first year of the study in 1999. The *Rhizoctonia* isolates were inoculated in equal quantities into a corn-meal/vermiculite media and cultured for 5 days. The corn-meal/vermiculite media subsequently became well established with actively growing *Rhizoctonia* mycelium. This then became the 'inoculum' and was amended to the surface soil.

The Treatments

	Treatments
1	Unamended (untreated) Control
2	Inoculum Level 1 - 250g of inoculum/m^2 soil
3	Inoculum Level 2 - 1Kg of inoculum/m^2 soil

Incorporation of Inoculum

The inoculum was added to the soil at 10 days after emergence of red beet seedlings. The *Rhizoctonia* was lightly incorporated into the soil between the beetroot rows.

Crop Diary

	Date
Drill Date	19 May 00
Inoculum incorporation	16 June 00
Final Harvest Assessment	12 September 00

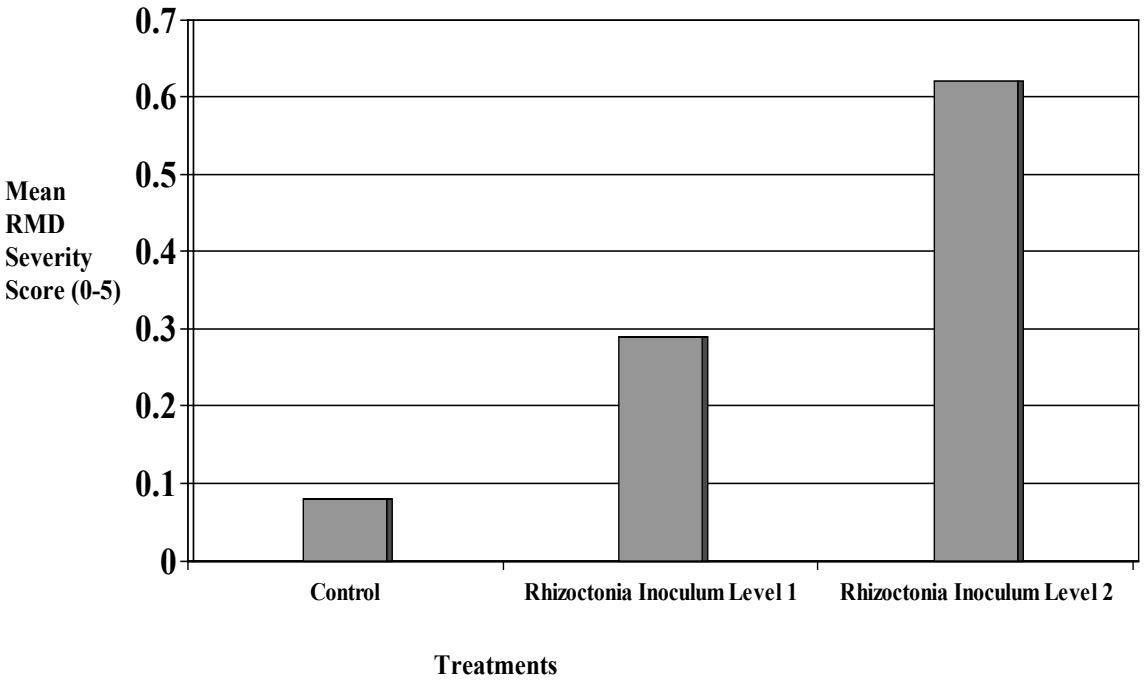
Assessments

The assessment of severity of root malformation was made using a 0-5 scale of RMD severity and was assessed at the final harvest. The chart used for assessing RMD severity is included in Appendix II. Assessments were performed on 50 roots randomly harvested from each plot.

3.3 Results

The results for mean RMD severity are displayed in the graph (histograms) on the following page and a table of the statistically analysis of these results is displayed in Appendix IV.

Figure 7: Mean RMD Severity in Red Beet Grown in Rhizoctonia Amended Field Soil at Two Concentrations



3.4 Discussion of Results

The results indicate that the *Rhizoctonia* fungal isolates previously detected on RMD affected red beet successfully caused root malformation on red beet plants when amended to the surrounding soil.

The higher level of inoculum caused the most significant root malformation with significantly more malformation than the control or the lower level of inoculum. A low level of RMD was detected in the untreated control plots and this was thought to be caused by wind-blown inoculum from the treated plots as it had been particularly wet and windy following amendment of the inoculum.

4. General Discussion

The work performed in this final year of the project focused on understanding the involvement of the three pathogens (*Rhizoctonia, Pythium* and *Aphanomyces*), that were implicated as the cause of RMD in the first year. The main thrust of the work was a series of field trials that were aimed at targeting the three pathogens and highlighting their involvement in the development of root malformation in red beet. The results indicated, by a process of elimination using seed treatments and drenches, that *Rhizoctonia* and *Pythium* are the organisms that are causing damage to seedlings and resulting in deformity of mature roots. The results also indicate that there was considerable variability between sites with either *Rhizoctonia* and/or *Pythium* the main cause of RMD depending on site and associated soil conditions. For example, the results from Site 1 showed that the control of *Rhizoctonia* by the use of Monceren as a seed treatment significantly reduced the occurrence of root malformation. There was also indication that *Pythium* was involved at this site with a response to SL567A demonstrated in the final harvest results. Biomex SA improved seedling vigour and thereby allowed seedlings to escape early attack from *Rhizoctonia*. At Site 2, *Pythium* was clearly implicated as the cause of RMD with very good control of root malformation with the use of SL567A (active against *Pythium*) both as a seed and drench treatment. The results from Site 3 are less conclusive; this site had slightly higher levels of RMD at the first (mid-season) assessment than at final harvest. It appeared that plants recovered producing a larger proportion of normal red beet than was anticipated earlier in the season. Early indications from this site implicate *Pythium* as a major factor in root malformation with SL567A reducing the numbers of RMD affected roots. At this Site, as in Site 1, Biomex SA also reduced the levels of RMD, although these results did not continue to harvest with low levels of RMD at the end of the season in this trial.

It appeared from the field trials that different conditions of soil moisture and topography are linked to the occurrence of *Rhizoctonia* and *Pythium* as the initiators of RMD. Site 1 being a drier and a well drained site was dominated by *Rhizoctonia,* an organism which has a wide range of environmental tolerances and can survive well in drier surface soils. Alternatively, Site 2 which was very wet throughout the season was dominated by the occurrence of *Pythium* as the cause of RMD.

It is well accepted that the development and spread of *Pythium* is encouraged by wet and poorly drained soils. It is therefore likely that these organisms are occurring either together and/or separately not only at different sites but also within the same field with their occurrence being dependent on local soil conditions of compaction and drainage.

The results for this years crop monitoring corroborate the evidence collected in the field trials. Both *Pythium* and *Rhizoctonia* were consistently detected either separately or together on red beet plants at the seedling stage. They were found in association with minute areas of damage on developing stems with seedlings often surviving early attack to develop areas of scar tissue which caused a resulting misshapen growth of the developing root. It was quite easy to see the very early stages of root malformation as these affected seedlings developed to produce miniature deformed roots at around 12 weeks post drilling. It was also apparent that the deformity produced by these organisms caused a slowing in growth of the developing root with roots become dense and woody as they overcame the scarring on the outside of the roots. The *Pythium* isolates collected from affected red beet in the crop monitoring were identified as *Pythium ultimum* which is the *Pythium* species implicated as part of a disease complex in red beet in the USA.

In confirmation of observations in the first year of the study, it was noticed during crop monitoring how RMD was more severe in compacted and poorly drained areas however raised and more sandy areas were also affected. It was apparent that *Rhizoctonia* was more regularly detected in affected seedlings in drier locations while *Pythium* and also *Rhizoctonia* were detected in wetter areas. It is therefore apparent that these two organisms are acting singly or as a disease complex as the cause of RMD in red beet.

The *Rhizoctonia* incorporation trial performed at Stockbridge House successfully demonstrated that the *Rhizoctonia* isolates, collected from RMD affected red beet in the first year of the study, could cause RMD symptoms when introduced to red beet seedlings. The symptoms produced in the red beet grown in *Rhizoctonia* amended soil produced the typical symptoms of russetting around the shoulder with clefting and bulbous protrusions.

The results of this year's study are supported by research from overseas that was highlighted in the literature search in the first year of this project. These reports from the United States (Abawi et al, 1974; Sherf & McNab, 1986; Olaya & Abawi, 1994) discuss a root rot complex caused by *Pythium ultimum* and *Rhizoctonia solani.* Under certain conditions of high soil moisture *Pythium ultimum* is seen as the primary pathogen with *Rhizoctonia solani* occasionally invading stem and root tissue already infected by *Pythium ultimum.* However under drier or warmer conditions *Rhizoctonia* was seen as the primary organism. The situation described in the States bears many similarities to that in the UK with plants being affected at the seedling stage by damping off, wirestemming and those seedlings, which survive earlier damage, become malformed.

5. Conclusions

- The results from the first year of the study indicated that the causes of RMD were initiated at the seedling stage during which damage to developing stems caused the formation of scar tissue, which subsequently restricted normal root growth.

- The causes of this early seedling damage were identified as being caused by infection by fungal organisms at the seedling stage. The involvement of other factors such as boron nutrition and virus infection were ruled out.

- Literature reviewed in the first year identified some key references which supported evidence collected from crop monitoring in the Eastern Counties that the organisms *Pythium*, *Rhizoctonia* and *Aphanomyces*, were involved in the early damage caused to stems and were possibly involved in the cause of RMD.

- A number of studies from the USA reported on a disease complex caused by *Pythium ultimum* and *Rhizoctonia solani* which produced a seedling blight and subsequent rotting and deformity in red beets. The symptoms described in this work were very similar to those seen in the UK.

- Information collected in the first year from red beet growers via distribution of a questionnaire confirmed the understanding that the increasing occurrence of wet Springs in recent years had been linked to the development of RMD.

- Seed treatment and red beet cultivar did not have any affect on the development of RMD with different crops being equally affected.

- Field trials performed in the second and final year of the study implicated both *Pythium* and *Rhizoctonia* as the causes of RMD, this observation was supported by crop monitoring also performed in the final year.

- *Aphanomyces* did not appear to be involved in the causes of root malformation but was involved in blackleg symptoms and damping-off of seedlings in non-Tachigaren treated crops.

- *Rhizoctonia* isolates collected in the first year from affected crops were successfully shown to cause root malformation when added to field soil in which red beet was grown.

- The wetter weather seen in the Spring season of recent years is directly exacerbating the build up of *Pythium* and *Rhizoctonia* inoculum in red beet soils.

- Differences in topography and soil conditions affect the distribution of *Pythium* and *Rhizoctonia* in RMD affected crops. *Pythium* primarily occurs in wet and less well dry sites whilst *Rhizoctonia* also occurs in wet and poorly drained but also drier and better drained locations.

- Improvements in seedling vigour appear to reduce the occurrence of RMD. This is apparent by the use of Biomex SA in the field trials, which reduced the occurrence of RMD at two sites. Also later sown crops, which avoided the wetter and cooler weather of Spring and produce a more vigorous seedling, appear to be generally less affected by RMD.

6. Technology Transfer

1. FV 226 Project Review – 6 January 2000.
2. HDC Annual Report – March 2000.
3. HDC Annual Report – March 2001.
4. HDC News – 'Red Beet Malformation'. Article in No. 69, January 2001.
5. Vegetable Farmer – 'Dealing with Root Malformation Disorder in Red Beet'. Article, March 2001.

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Jeffers, S.N. and Martin, S.B. (1986). Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Disease, 70:1038-1043.

Olaya, G. and Abawi, G.S. (1994). Influence of inoculum type and moisture on development of *Rhizoctonia solani* on foliage and table beets. Plant Disease, 78:8, 805-810.

Pfender, W.F., Delwich, P.A., Grau, C.R. and Hagedorn, D.J. (1984). A medium to enhance recovery of *Aphanomyces* from infected plant tissue. Plant Disease, 68:845-847.

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Sherf, A. F. & Macnab, A. A. (1986). Vegetable Diseases and Their Control. John Wiley & Sons, New York. 728pp.

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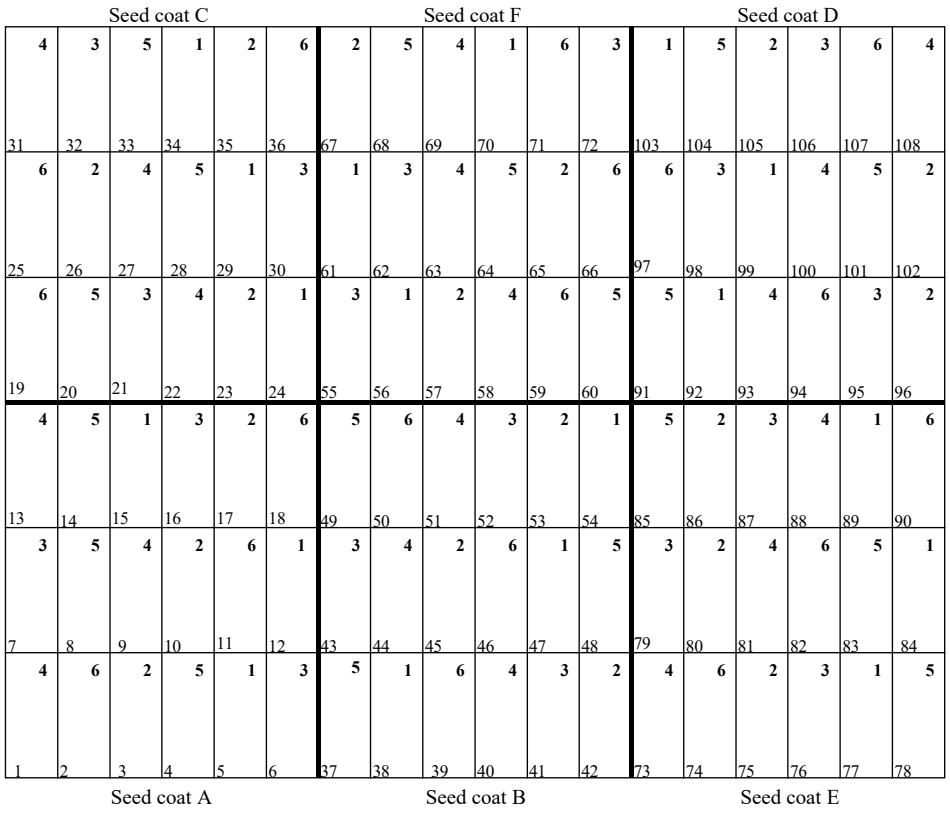
Thankyou also to Feli Pomares and Cathryn Lambourne for technical support in all aspects of the work.

Appendix I:

Trial Plans - Site 1 Spilspy

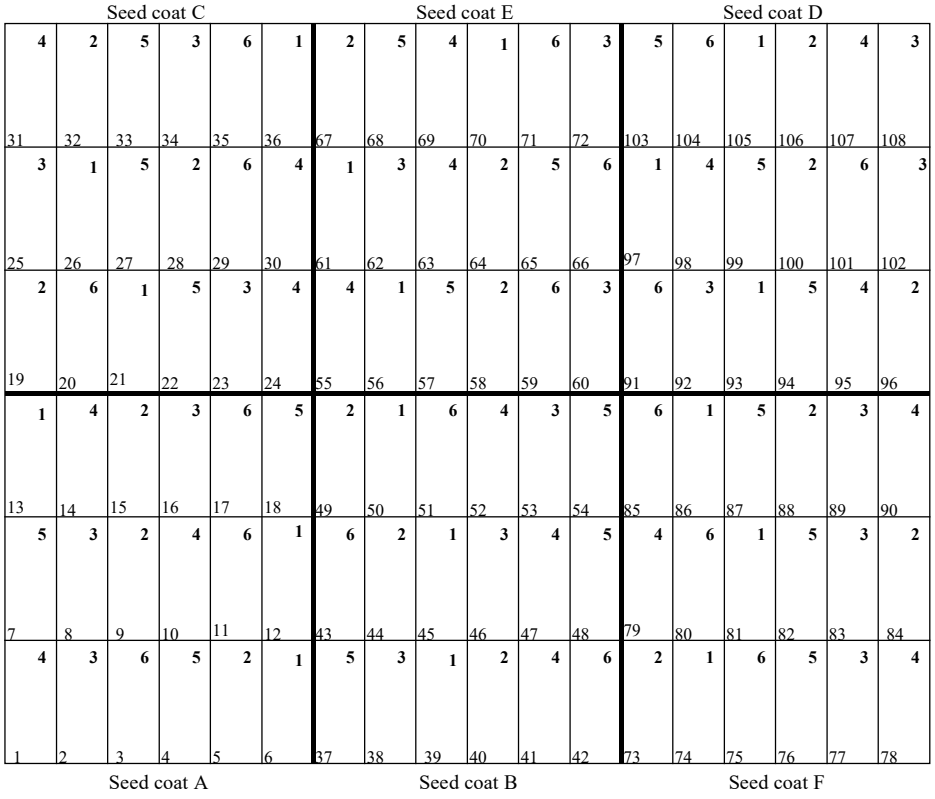


Seed Treatment:
A: Control (Thiram only)
B: SL567A
C: Tachigaren
D: Monceren
E: Biomex SA
F: SL567A + Monceren

Post Emergence Drench:
1: Untreated Control
2: SL567A
3: Tachigaren
4: Basilex
5: Amistar
6: Biomex SA

Plot size:
3m x 1.83m

Trial Plans - Site 2 Westwoodside

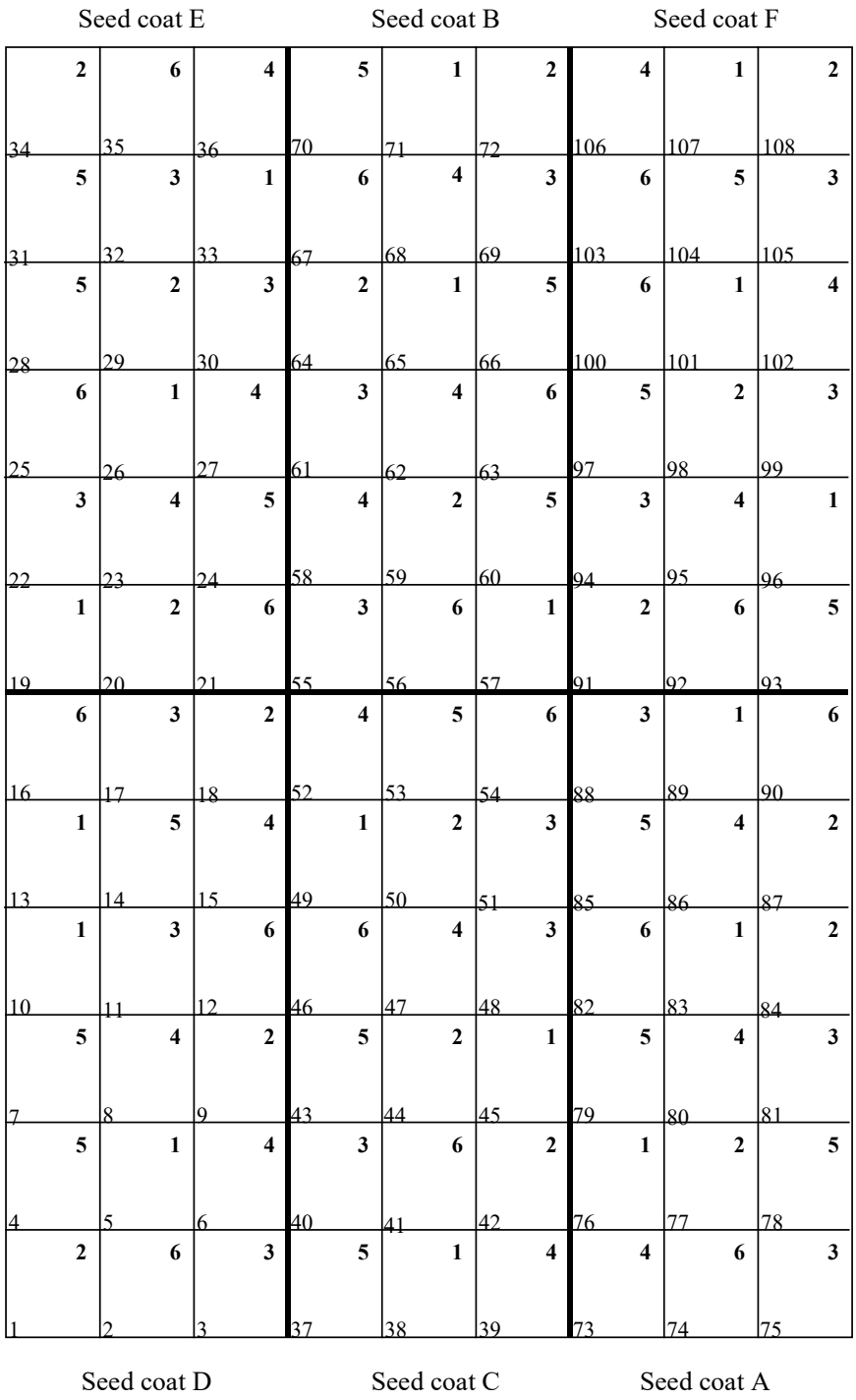


Seed Treatment:
A: Control (Thiram only)
B: SL567A
C: Tachigaren
D: Monceren
E: Biomex SA
F: SL567A + Monceren

Post Emergence Drench:
1: Untreated Control
2: SL567A
3: Tachigaren
4: Basilex
5: Amistar
6: Biomex SA

Plot size:
4m x 1.83m

Trial Plans – Site 3 West Butterwick



Seed Treatment:
A: Control (Thiram only)
B: SL567A
C: Tachigaren
D: Monceren
E: Biomex SA
F: SL567 + Monceren

Post Harvest Drench:
1: Untreated Control
2: SL567A
3: Tachigaren
4: Basilex
5: Amistar
6: Biomex SA

Plot size:
4.1m x 1.83m

Appendix II: Root Malformation Disorder - Severity Assessment Chart

Appendix III:

Replicated Fungicide Trials – Tables of Results

Table 1: Site 1 - Severity of Root Malformation[a] in 50 Root Sample at First Harvest (9 August 00)

Seed Treatments		Drench Treatments					
		1 Control	2 SL567A	3 Tachigaren	4 Basilex	5 Amistar	6 Biomex SA
A	Untreated Control – Thiram (Thiram 600g/l) soaked (standard)	0.636	0.621	0.809	0.774	0.629	0.659
B	Thiram (Thiram 600g/l) soaked and SL567A (metalaxyl–M 2ml product/Kg seed) coated	0.484	0.687	0.599	0.492	0.569	0.498
C	Thiram (Thiram 600g/l) soaked and Tachigaren (hymexazol – 21g product/Kg seed) coated	0.485	0.545	0.490	0.571	0.476	0.516
D	Thiram (Thiram 600g/l) soaked and Monceren Flowable (pencycuron - 16ml product/Kg seed) coated	0.294	0.349	0.277	0.239	0.350	0.313
E	Scarified (acid treated) and Biomex SA (Trichoderma from Omex - 15ml/Kg seed) soaked	0.180	0.446	0.400	0.444	0.262	0.421
F	Thiram (Thiram 600g/l) soaked and Monceren Flowable (pencycuron - 16ml product/Kg seed) and SL567A (metalaxyl–M - 2ml product/Kg seed) coated	0.362	0.501	0.310	0.397	0.357	0.420
Seed Treatments LSD 5% (12df)		0.23					
Seed Treatment Blocks – Variance Ratio		3.66 (Critical Value - F distribution at 5/12 df = 3.11)					
Drench Treatments Significance		NS					
Drench Treatments LSD 5% (60df)		0.11					

[a] The results for severity of RMD have been square root transformed to homogenise variance.

Table 2: Site 1 - Severity of Root Malformation[a] in 50 Root Sample at Final Harvest (25 September 00)

Seed Treatments		Drench Treatments					
		1 Control	2 SL567A	3 Tachigaren	4 Basilex	5 Amistar	6 Biomex SA
A	Untreated Control – Thiram (Thiram 600g/l) soaked (standard)	0.549	0.641	0.637	0.533	0.410	0.681
B	Thiram (Thiram 600g/l) soaked and SL567A (metalaxyl–M 2ml product/Kg seed) coated	0.427	0.345	0.437	0.574	0.399	0.575
C	Thiram (Thiram 600g/l) soaked and Tachigaren (hymexazol – 21g product/Kg seed) coated	0.628	0.413	0.445	0.732	0.539	0.363
D	Thiram (Thiram 600g/l) soaked and Monceren Flowable (pencycuron - 16ml product/Kg seed) coated	0.325	0.321	0.288	0.288	0.228	0.321
E	Scarified (acid treated) and Biomex SA (Trichoderma from Omex - 15ml/Kg seed) soaked	0.532	0.307	0.515	0.541	0.309	0.413
F	Thiram (Thiram 600g/l) soaked and Monceren Flowable (pencycuron - 16ml product/Kg seed) and SL567A (metalaxyl–M - 2ml product/Kg seed) coated	0.219	0.410	0.425	0.410	0.393	0.215
Seed Treatments LSD 5% (12df)		0.25					
Seed Treatment Blocks – Variance Ratio		1.06 (Critical Value - F distribution at 5/12 df = 3.11)					
Drench Treatments Significance		*					
Drench Treatments LSD 5% (60df)		0.13					

[a] The results for severity of RMD have been square root transformed to homogenise variance.

Table 3: Site 2 - Severity of Root Malformation[a] in 50 Root Sample at First Harvest (15 August 00)

Seed Treatments		Drench Treatments					
		1 Control	2 SL567A	3 Tachigaren	4 Basilex	5 Amistar	6 Biomex SA
A	Untreated Control – Thiram (Thiram 600g/l) soaked (standard)	0.922	0.859	0.767	0.845	0.787	0.764
B	Thiram (Thiram 600g/l) soaked and SL567A (metalaxyl–M 2ml product/Kg seed) coated	0.762	0.651	0.814	0.820	0.679	0.691
C	Thiram (Thiram 600g/l) soaked and Tachigaren (hymexazol – 21g product/Kg seed) coated	0.774	0.745	0.791	0.907	0.875	1.003
D	Thiram (Thiram 600g/l) soaked and Monceren Flowable (pencycuron - 16ml product/Kg seed) coated	0.675	0.603	0.676	0.671	0.803	0.725
E	Scarified (acid treated) and Biomex SA (Trichoderma from Omex - 15ml/Kg seed) soaked	0.858	0.613	0.810	0.740	0.806	0.651
F	Thiram (Thiram 600g/l) soaked and Monceren Flowable (pencycuron - 16ml product/Kg seed) and SL567A (metalaxyl–M - 2ml product/Kg seed) coated	0.658	0.635	0.644	0.644	0.595	0.675
Seed Treatments LSD 5% (12df)		0.17					
Seed Treatment Blocks – Variance Ratio		2.3 (Critical Value - F distribution at 5/12 df = 3.11)					
Drench Treatments Significance		NS					
Drench Treatments LSD 5% (60df)		0.08					

[a] The results for severity of RMD have been square root transformed to homogenise variance.

Table 4: Site 2 - Severity of Root Malformation[a] in 50 Root Sample at Final Harvest (3 October 00)

Seed Treatments		Drench Treatments					
		1 Control	2 SL567A	3 Tachigaren	4 Basilex	5 Amistar	6 Biomex SA
A	Untreated Control – Thiram (Thiram 600g/l) soaked (standard)	1.174	0.749	1.362	1.066	1.111	1.050
B	Thiram (Thiram 600g/l) soaked and SL567A (metalaxyl–M 2ml product/Kg seed) coated	0.269	0.365	0.407	0.324	0.421	0.352
C	Thiram (Thiram 600g/l) soaked and Tachigaren (hymexazol – 21g product/Kg seed) coated	1.426	0.648	1.441	1.549	1.392	1.480
D	Thiram (Thiram 600g/l) soaked and Monceren Flowable (pencycuron - 16ml product/Kg seed) coated	0.963	0.540	0.759	1.194	0.807	1.096
E	Scarified (acid treated) and Biomex SA (Trichoderma from Omex - 15ml/Kg seed) soaked	1.457	0.393	1.101	0.923	0.925	1.212
F	Thiram (Thiram 600g/l) soaked and Monceren Flowable (pencycuron - 16ml product/Kg seed) and SL567A (metalaxyl–M - 2ml product/Kg seed) coated	0.373	0.325	0.376	0.318	0.245	0.498
Seed Treatments LSD 5% (12df)		0.22					
Seed Treatment Blocks – Variance Ratio		31.06 (Critical Value - F distribution at 5/12 df = 3.11)					
Drench Treatments Significance		***					
Drench Treatments LSD 5% (60df)		0.14					

[a] The results for severity of RMD have been square root transformed to homogenise variance.

Table 5: Site 3 - Severity of Root Malformation[a] in 50 Root Sample at First Harvest (15 August 00)

Seed Treatments		Drench Treatments					
		1 Control	2 SL567A	3 Tachigaren	4 Basilex	5 Amistar	6 Biomex
A	Untreated Control – Thiram (Thiram 600g/l) soaked (standard)	0.887	0.784	0.762	0.957	0.769	0.914
B	Thiram (Thiram 600g/l) soaked and SL567A (metalaxyl–M 2ml product/Kg seed) coated	0.606	0.516	0.631	0.637	0.596	0.596
C	Thiram (Thiram 600g/l) soaked and Tachigaren (hymexazol – 21g product/Kg seed) coated	0.926	0.638	0.776	0.775	0.823	0.743
D	Thiram (Thiram 600g/l) soaked and Monceren Flowable (pencycuron - 16ml product/Kg seed) coated	0.909	0.738	0.844	0.851	0.940	0.921
E	Scarified (acid treated) and Biomex SA (Trichoderma from Omex - 15ml/Kg seed) soaked	0.643	0.480	0.595	0.647	0.390	0.451
F	Thiram (Thiram 600g/l) soaked and Monceren Flowable (pencycuron - 16ml product/Kg seed) and SL567A (metalaxyl–M - 2ml product/Kg seed) coated	0.617	0.581	0.716	0.637	0.605	0.570
Seed Treatments LSD 5% (12df)		0.15					
Seed Treatment Blocks – Variance Ratio		7.25 (Critical Value - F distribution at 5/12 df = 3.11)					
Drench Treatments Significance		*					
Drench Treatments LSD 5% (60df)		0.09					

[a] The results for severity of RMD have been square root transformed to homogenise variance.

Table 6: Site 3 - Severity of Root Malformation[a] in 50 Root Sample at Final Harvest (26 September 00)

Seed Treatments		Drench Treatments					
		1 Control	2 SL567A	3 Tachigaren	4 Basilex	5 Amistar	6 Biomex SA
A	Untreated Control – Thiram (Thiram 600g/l) soaked (standard)	0.604	0.465	0.721	0.523	0.419	0.699
B	Thiram (Thiram 600g/l) soaked and SL567A (metalaxyl–M 2ml product/Kg seed) coated	0.560	0.279	0.504	0.386	0.417	0.421
C	Thiram (Thiram 600g/l) soaked and Tachigaren (hymexazol – 21g product/Kg seed) coated	0.570	0.348	0.756	0.370	0.351	0.383
D	Thiram (Thiram 600g/l) soaked and Monceren Flowable (pencycuron - 16ml product/Kg seed) coated	0.590	0.325	0.341	0.410	0.413	0.403
E	Scarified (acid treated) and Biomex SA (Trichoderma from Omex - 15ml/Kg seed) soaked	0.520	0.287	0.292	0.316	0.258	0.443
F	Thiram (Thiram 600g/l) soaked and Monceren Flowable (pencycuron - 16ml product/Kg seed) and SL567A (metalaxyl–M - 2ml product/Kg seed) coated	0.326	0.239	0.408	0.296	0.197	0.243
Seed Treatments LSD 5% (12df)		0.02					
Seed Treatment Blocks – Variance Ratio		1.84 (Critical Value of F distribution at 5/12 df = 3.11)					
Drench Treatments Significance		NS					
Drench Treatments LSD 5% (60df)		0.12					

[a] The results for severity of RMD have been square root transformed to homogenise variance.

Appendix IV:

Table 7: Rhizoctonia Incorporation Trial – Severity of Root Malformation in 50 Root Sample

Treatments	Mean RMD Severity Score
Control (unamended)	0.08
Rhizoctonia Inoculum Level 1 250gm/m2	0.28
Rhizoctonia Inoculum Level 2 1kg/m2	0.62
Significance	*
LSD (5% 6df)	0.34