Project title	Red beet: further elucidation of the cause, epidemiology and control of root malformation disorder (RMD) on red beet
Project number	FV 226a
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Report	Preliminary report 2003
Previous reports	None
Date project commenced	1 November 2002

Headline

- This preliminary study supported the previous project (FV 226) which demonstrated that SL567A helped to control RMD.
- A SOLA was obtained and distributed for SL567A in June 2003.
- Further R&D looking at the cause of RMD is now ongoing.

Introduction

Following the onset of commercial harvesting of the UK Red Beet crop in October 2002 it quickly became apparent that the disorder referred to as RMD (Root Malformation Disorder) was highly prevalent and damaging in some crops, especially in the Isle of Axholme region of South Yorkshire. In some cases entire fields were unmarketable due to the severity of the root disorder. Progress on grading lines was extremely slow as the distorted beet had to be separated out by hand and this significantly increased production costs and severely delayed progress with the harvesting process. This was further compounded by rejections by the processors due to the presence of misshapen beet in individual consignments delivered to the factory.

An urgent site visit to commercial crops (Mr G Smith, Westwoodside, Doncaster) was made by STC personnel and the severity of the 2003 problem was quickly established. Previous HDC-funded studies (FV 226 : 1999-2000) implicated Pythium and Rhizoctonia as the causal organisms of RMD. However, the pattern and spread of disease in 2002 suggested an air-borne source may also be involved.

A meeting comprising red beet growers, processors and other stakeholders was held at STC in early November under the auspices of the Red Beet Technology Group. The findings of the previous study (FV 226) were discussed, other potential causal agents discussed and plans formulated to extend R&D, initially via emergency funding during the over-wintering period, with a view to more extensive field-scale trials commencing in Spring 2003. The preliminary studies focused on attempts to determine whether the problem was caused primarily by a soil-borne pathogens eg *Pythium, Rhizoctonia* or an air-borne organism e.g. downy mildew or pests eg aphids vectoring an as yet unidentified virus.

Various approaches were taken in a further bid to elucidate the primary cause(s) for this unusual root distortion problem afflicting red beet.

1. **Investigation of RMD affected sites *in situ***
2. **Pot study to evaluate soil sterilisation & various fungicide applications**
3. **Pot study to investigate the potential role of downy mildew in the aetiology of RMD**
4. **Investigation of the potential for viruses causing RMD symptoms in red beet**

This brief report therefore provides a summary report of progress to date with the initial over-wintering investigations aimed at elucidating the cause of the root malformation disorder.

1. Investigation of RMD affected sites *in situ*

An initial site visit to affected crops in the Isle of Axholme region of South Yorkshire quickly highlighted the devastating effects RMD was having at some sites. At one 20 acre site (G Smith, personal communication) around 95% of beet were affected with symptoms of RMD though scattered randomly within the field it was still possible to find occasional roots unaffected.

Of particular note was the high level of leaf senescence and petiole blackening. In some cases this was also associated with distorted re-growth of leaf tissues in the crown area. A significant proportion of these plants (ca. 25-35%) exhibited sporulation of *Peronospora farinosa*, cause of downy mildew in red beet. However, a large proportion of the affected plants exhibited no apparent sporulation by this obligate parasite. The incidence of rust (*Uromyces betae*) was also high, though this was present on affected and unaffected plants at the same level. A further visit was made to the affected sites some 2-3 weeks later to collect soil & plant samples for further study.

In follow-up investigations in the scientific literature it became apparent that there were reports of *P. farinosa* causing systemic infection under certain environmental conditions in other crops. This phenomenon is also reported to occur with other d. mildew pathogens. In one unpublished report (Oregon, USA; 1977) downy mildew is reported to have been responsible for severe malformation of table beet, the symptoms of which appear to be very similar to those of RMD.

Contact was made with researchers in Oregon though ultimately no further useful information could be gleaned though the possibility of systemic d. mildew as a potential cause for RMD requires further investigation. It should be noted that as an obligate parasite it cannot be grown in artificial culture media and therefore all studies have to be conducted using living host tissues (*in planta*). Evidence in other d. mildew pathogens suggests that the organism can, in some situations, be both seed- and soil-borne.

2. Pot study to evaluate soil sterilisation & various fungicide applications

Soil samples from a field badly affected by RMD (32 acre field, G Smith) were collected on the 25 October 2002, along with soil from a relatively unaffected field.

The soil from the badly affected field was divided into two and one half was autoclaved at 121°C for 15mins at 1 Bar pressure. The two batches of soil were then used to set up a replicated pot trial sown with red beet seed at two densities. Depending on the outcome this approach could determine whether the root distortion seen in the field was caused by a soil-borne rather than an air-borne pest/pathogen. A series of fungicide treatments was superimposed over the sterilised/non-sterilised pots to gain further information regarding the potential primary cause.

Each of the soil batches (sterilised and unsterilised) was used to fill 48 x 30cm pots. Each treatment comprised 8 pots. Four of these 8 were sown with 15 seeds and 4 with 30 seeds per pot to determine whether seedling density played a part in the occurrence of the disorder. A range of 5 fungicides was applied as a pre-emergent drench and also as a drench following germination.

The soil from the relatively unaffected field was used to fill 18 x 30cm pots, which received the same fungicide treatments as the larger pot trial described above. These pots provided a useful comparison with the unsterilised affected soil.

The Treatments

Treatment	Active Ingredient	Target	Rate
Control	-	-	-
SL567A	metalaxyl-M	Oomycete fungi	1ml/litre*
Monceren	pencycuron	*Rhizoctonia*	1ml/litre
Tachigaren	hymexazol	*Aphanomyces*	1g/litre
Bavistin	carbendazim	*Phoma*	1g/litre
Amistar	azoxystrobin	Broad spectrum	1ml/litre

* Reduced to 0.5ml/litre for the post-emergence application.

Red Beet seed New Globe (S&G) thiram soaked was used throughout.

The pre-emergent drench was applied on the 18 November 2002. The post-emergent drench was applied on the 21 January 2003.

Treatments were applied as a 50ml/pot drench.

Results

Summary of germination counts/treatment

Table 1. Unsterilised 'Affected' Soil

Treatment	Mean percentage healthy seedlings*			
	4/12/02	11/12/02	18/12/02	03/01/03
Control	37.2	39.4	38.9	15.5
SL567A	58.9	58.9	46.1	25.0
Monceren	53.9	45.5	28.9	10.0
Tachigaren	57.2	51.7	45.0	31.7
Bavistin	53.3	38.3	34.4	29.4
Amistar	40.0	26.1	25.0	15.0

** Mean of all plots assessed*

N.B. Numbers shown in red indicate the mean highest percentage germination to date

Table 2. Sterilised 'Affected' Soil

Treatment	Mean percentage healthy seedlings*			
	4/12/02	11/12/02	18/12/02	03/01/03
Control	64.4	61.1	61.1	56.7
SL567A	45.0	42.2	40.0	2.8
Monceren	37.7	46.7	40.0	47.8
Tachigaren	43.3	72.8	70.5	56.7
Bavistin	54.4	49.4	53.3	51.1
Amistar	31.7	37.8	37.8	37.8

** Mean of all plots assessed*

N.B. Numbers shown in red indicate the mean highest percentage germination to date

Table 3. Unsterilised 'Unaffected' Soil

Treatment	Mean percentage healthy seedlings*			
	4/12/02	11/12/02	18/12/02	03/01/03
Control	38.9	46.7	46.7	40.0
SL567A	36.7	42.2	32.2	24.4
Monceren	55.5	55.5	56.7	51.1
Tachigaren	61.1	65.5	60.7	48.8
Bavistin	55.6	62.2	63.3	52.2
Amistar	51.1	50.0	56.7	43.3

** Mean of all plots assessed*

N.B. Numbers shown in red indicate the mean highest percentage germination to date

The most marked effect observed in the pot study was that there was a decreased incidence of post-emergence (and possibly pre-emergence) damping-off in the non-sterilised soil as compared with the autoclaved soil. This indicates, perhaps not unexpectedly, the presence of soil-borne fungi in the untreated field soil which could potentially impact on seedling vigour post-drilling. Attempts to identify the primary cause of the damping-off in follow-up laboratory tests were somewhat inconclusive though both *Pythium* and *Rhizoctonia* were recovered from occasional collapsed seedlings. The incidence of *Aphanomyces*, *Phoma* and other known pathogens of *Beta* species appeared to be negligible in these isolation tests.

Distribution of the maximum seedling numbers (as presented in red in Tables 1-3) in each treatment and 'soil type' shows an interesting pattern. The maximum emergence in the 'affected', but sterilised, soil occurred early (Table 2). In contrast the maximum emergence in the same, but unsterilised, soil was delayed and this suggested that a degree of pre-emergence infection was also taking place in this soil.

Following repeated applications of the various fungicide treatments seedling viability appeared to be affected relative to the untreated control. SL567A, for some unaccountable reason, caused some scorching of the seedling leaves following emergence and this got progressively worse as the experiment progressed even though the rate of application of the product was reduced by 50% for the repeat treatment. The effect was most marked in the autoclaved soil and this suggests it was not due to an unusual interaction with soil-borne pathogens.

Treatment with Amistar also appeared to adversely affect plant stand in the experiment as seedling numbers in the sterilised and unsterilised soil were reduced following fungicide application and this persisted throughout the experiment.

In assessments conducted soon after emergence all the fungicide treatments applied to the untreated soil (Table 1) appeared to increase plant stand relative to the untreated control. SL567A was most effective in this regard suggesting that an oomycete fungus was having the greatest impact on seedling vigour. This could be due to a number of pathogens including *Pythium*, *Peronospora* , *Phytophthora* or possibly even *Aphanomyces*. Unfortunately the occurrence of phytotoxicity symptoms following treatment with SL567A prevented further interpretation of data from this treatment.

In the same, but sterilised, soil (Table 2) none of the treatments provided an initial plant stand equivalent to the untreated control and this suggested that all the fungicide treatments had a slight detrimental effect on germination of the beet seed. However, by the second assessment on 11 December treatment with Tachigaren appeared to provide a marked improvement in seedling establishment. This result is difficult to explain as soil sterilisation would have eliminated all propagules of the soil-borne pathogen *Aphanomyces* which is the primary target for this fungicide.

In the unsterilised, but unaffected, soil most of the fungicides, with the exception of SL567A, improved seedling emergence by the first assessment on 4 December (Table 3). The poor result with SL567A can again be accounted for by an apparent phytotoxic reaction. The overall seedling survival in this untreated unaffected soil was much lower than that observed in the untreated affected soil (Table 1) and this suggests a much lower inoculum of soil-borne pathogens in the field site. This may be of significance relative to the incidence of RMD in these two field sites.

The observation of the pots is still on going. It is hoped that as the seedlings begin to develop swollen roots some evidence of RMD may be seen in some of the seedlings in the unsterilised ‘affected’ soil.

Conclusions

- A clear difference in seedling viability was seen between the unsterilised and sterilised ‘affected’ soil.
- Increased damping-off was observed in the ‘affected’ soil compared with the ‘unaffected’ soil.
- Both *Pythium spp*. and *Rhizoctonia spp*. were isolated from seedlings damping-off in the unsterilised ‘affected’ soil.
- *Aphanomyces* and *Phoma* were only detected at negligible levels on the damped-off seedlings.
- In the unsterilised ‘affected’ soil initial seedling emergence was improved slightly in all treatments but particularly SL567A & Tachigaren.

- In the sterilised ‘affected’ soil initial seedling emergence was not improved by any of the applied fungicides though in later assessments Tachigaren treatment provided the highest level of seedling viability.

- SL567A at the higher rate of 1ml/litre resulted in phytotoxicity symptoms, which over time, masked treatment effects which may have been present.

- A lower incidence of damping-off was noted in the ‘unaffected’ field soil and this correlates well with a lower incidence of RMD at this site.

- The remaining seedlings will be retained in the pots for a further period to ascertain whether RMD symptoms develop in the Spring.

3. Pot study to investigate the potential role of downy mildew in the aetiology of RMD

Red Beet severely affected by RMD were collected from a badly affected field site in the Isle of Axholme. Affected roots were potted up in standard compost, 4 roots/pot into a total of 10 pots. Downy mildew spores from infected leaves collected from the same field site were used to inoculate all the other plants. The trial was located in an unheated polytunnel.

Two replicate pots/treatment were sprayed with a range of fungicides at fortnightly intervals for a total of 4 applications in an attempt to prevent further RMD symptoms occurring.

The treatments

Treatment	Active ingredient	Rate of use
Untreated (water)	-	-
SL567A	metalaxyl-M	1ml/litre
Amistar	azoxystrobin	1ml/litre
Invader	dimethomorph + mancozeb	1g/litre
Monceren	pencycuron	1ml/litre

Treatments were applied with a hand sprayer and applied to run-off.

Results

By the time of this report downy mildew had established on a small no. of the untreated plants though no infection was evident of the fungicide treated plots. Further observation of this trial will be undertaken over time to further elucidate any possible role played by this pathogen in the potential systemic invasion of red beet plants and its capacity to cause root distortion symptoms following such systemic infection.

Parallel attempts to establish d. mildew on young beet seedlings have been unsuccessful due to the continued damping-off of the beet seedlings during emergence. It is hoped there will be a further opportunity to repeat this work in the Spring when environmental conditions are more favourable.

4. Investigation of the potential for viruses causing RMD symptoms in red beet

Samples of RMD affected and apparently unaffected roots were forwarded to CSL for virus testing. The samples were tested by ELISA for *Beet necrotic yellow vein virus* (BNYVV). No virus was detected. Electron microscopy of the samples did not reveal any virus particles. Further EM and ELISA work was also carried out to check for the presence of *Tomato blackring virus* (TBRV) and *Tobacco necrosis virus* (TNV). However, once again these tests proved negative.

Following sap inoculations from the healthy and RMD affected beet samples onto a range of indicator plants local lesion symptoms were detected in *Nicotiana benthamiana.* Initially, this seemed to indicate the presence of a possible virus cause. However, similar symptoms were also observed in plants inoculated with sap from apparently healthy beet. Further investigation and isolations from the local lesions detected on the indicator plants were subsequently carried out by CSL personnel. The bacterium *Pseudomonas syringae* (pathovar not determined) was isolated from the local lesions and regarded to be responsible for the localised symptom seen in the indicator plants.

In conclusion, no viruses were detected which could potentially be responsible for the root distortion in RMD affected crops.