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AUTHENTICATION

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

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Grower Summary

Headline

- 'Custo-Fume' (chloropicrin) soil treatment increased growth of *Sorbus aucuparia* and greatly reduced graft bud failure in trees grown on land affected by *Sorbus* replant disease.
- 'Agralan Revive' (*Bacillus subtilis*), 'Basamid' (dazomet), 'Biofence' (brassica seed meal), extra nitrogen (calcium nitrate), 'Novozymes Roots' (mycorrhiza) and 'PlantMate WP' (*Trichoderma harzianum*) treatments were ineffective.
- Tests on roots and soil indicate *Pythium* species are likely causal agents of *Sorbus* replant disease.

Background and expected deliverables

Replant diseases of ornamental rosaceous trees are complex soil-borne problems. Fungi and actinomycete bacteria appear to be the most likely cause. There is evidence that even on a single host species more than one microorganism may be involved and the causes may differ between sites.

Previous work on replant diseases of rose and ornamental rosaceous trees is summarised in HNS 152, covering symptoms, diagnosis, causes, disease management and control. The overall aim of this project was to reduce losses to replant diseases in rosaceous trees through the use of control methods other than synthetic broad-spectrum soil biocides. The specific objectives were to:

- Develop a *Sorbus* seedling bioassay in order to assess soils for the risk of replant disease when this species is grown.
- Determine the efficacy of some biological and nutritional treatments in overcoming replant disease in *Sorbus*.

Summary of the project and main conclusions

Overview of the seedling bioassay

A seedling bioassay to test soils for the presence of Specific Apple Replant Disease (SARD) was originally developed in the Netherlands in the 1960s. This test and variations of it have been used to determine the cost: benefit of disinfesting a soil before planting apple and to investigate treatments for control of SARD.

Briefly, a SARD seedling bioassay involves measurement of the growth response to soil disinfestation with a fumigant. In the ADAS SARD test, soil was collected in the spring from 0-30 cm depth from the prospective planting site. One half of the sample was treated with the fumigant 'Custo-Fume' (chloropicrin) (2.5 ml/10 L soil) and the remainder left untreated. After dissipation of fumes, ten pots were filled with the treated and ten with untreated soil and planted with apple seedlings, cv. Bittenfelder. The pots were placed in cold frames and plant height was measured after 12 weeks. Percentage growth response (R) was calculated from the formula %R = 100 F/U where F and U are the mean heights of plants in fumigated and untreated soil. Field fumigation was considered economically beneficial for rootstocks sensitive to SARD if the percentage growth response was 150-199%, and for all rootstock types if the response was over 200%.

Development of a *Sorbus* seedling bioassay

In 2008 an experiment was devised to determine if a pot test using *Sorbus aucuparia* seedlings could differentiate soils with different cropping histories. Two methods of soil disinfestation were compared. Soil was collected from around the base of three year old *Sorbus aucuparia* trees (designated 'high risk' of *Sorbus* replant disease) and from a field where no rosaceous crops had been grown for at least 20 years (designated 'low risk' of *Sorbus* replant disease). Soils were treated with 'Custo-Fume' (chloropicrin) at the equivalent of 280 L/ha, or autoclaved (steam sterilized) twice, or left untreated. Seedling height was measured at intervals after transplanting and the percentage growth response (R) calculated (Table 1).

Table 1: Percentage growth response (R) of *Sorbus* seedlings, calculated on increase in height, in soils considered to differ in risk of *Sorbus* replant disease

Assessed risk of *Sorbus* replant disease based on cropping history	Soil treatment	% R after	
		7 weeks	11 weeks
'Low risk'	Autoclave	143	**171**
	Custo-Fume	122	142
'High risk'	Autoclave	**198**	**238**
	Custo-Fume	**169**	**153**

Percentage growth response values of 150-199 predict a moderate risk of replant disease and over 200 predict a severe risk of replant disease. Soils at risk of moderate or severe replant disease are shown in bold.

Soil treatment had no significant effect on seedling height until 7 weeks after transplanting. At 11 weeks after transplanting, percentage growth response clearly differentiated the two soils by replant disease severity category irrespective of method of soil treatment. The

results of this experiment suggest that a bioassay test duration of at least 11 weeks is preferred.

Autoclave treatment of the soils consistently resulted in a larger growth response than 'Custo-Fume' treatment. At the 11 week assessment, autoclave treatment of the 'low risk' soil resulted in a growth response of more than 150; i.e. the test predicted moderate replant disease when none was expected, presumably a false positive result. For this reason, and because chloropicrin is currently the method most commonly used for field treatment of replant disease in the UK, 'Custo-Fume' was used to treat soils in future bioassay tests.

A mixture of fungi was recovered from roots of *Sorbus* seedlings grown in the 'high risk' soil, including species of *Cylindrocarpon*, *Fusarium* and *Pythium*. These fungi have previously been implicated as causes of replant disease of other rosaceous crops. By contrast, only a *Trichoderma* species was consistently isolated from roots of *Sorbus* seedling grown in the same soil after 'Custo-Fume' treatment.

Test of the *Sorbus* seedling bioassay (Experiment 1)

In early summer 2009, seven soils predicted to differ in their level of *Sorbus* replant disease were tested using the bioassay method developed. Soil collected from around two-year-old *Sorbus* trees on land untreated with chloropicrin was considered very high risk (site 1), soil collected from a site where no rosaceous species had been grown for at least 20 years was considered very low risk (site 7). Five other soils were classed at intermediate levels (Table 2).

The percentage growth response (R) to 'Custo-Fume' treatment of the soil was calculated based on increase in height, increase in weight of top growth and increase in weight of root growth. The extent of the growth response varied with soil source, indicating that the soils differed in their levels of *Sorbus* replant disease severity, as was expected (Table 2). Five of the seven soils resulted in a growth response in broad agreement with the predicted risk. The percentage growth responses calculated on weight of top growth and weight of root growth were largely in agreement with the traditional measure, increase in height.

Table 2: Effect of soil source on *Sorbus* replant disease severity as measured by increase in seedling height in a pot bioassay – Experiment 1, 2009

Soil Source	Site history			Predicted replant severity	Measured replant disease severity (% R and interpretation)	
	Current *Sorbus*	Recent *Sorbus*	Recent CP treatment			
1.	✓	-	-	Very high	307	High
2.	✓	-	✓	High	208	Moderate
3.	-	-	-	Moderate	174	Moderate
4.	-	-	✓	Low	385	High
5.	-	Uncertain	-	Uncertain	100	Low
6.	-	-	-	Low	79	Low
7.	-	-	-	Very low	108	Low

The reason for non-agreement of predicted and measured replant disease severity at Soil Source 4 is unknown. The soil was collected from around *Acer* tree roots grown on land treated with chloropicrin in 2005. There is no reported association between *Acer* trees and *Sorbus* replant disease. The existence of non-agreement means that the bioassay is not sufficiently reliable to offer as a commercial service.

Use of a *Sorbus* seedling bioassay to select field areas with contrasting replant risk (Experiment 2)

In summer 2009, five soils from a farm in Worcestershire were tested by the *Sorbus* seedling pot bioassay in order to identify two areas with contrasting *Sorbus* replant disease severity risk. This was done in preparation for a field experiment (Experiment 3) for evaluation of treatments to manage *Sorbus* replant disease. The two areas chosen as low risk and high risk areas for the field experiment were from a headland and from an in-field area, with R values of 95 and 207 respectively. The headland had not been cropped for at least 20 years; the in-field area grew *Sorbus* in 2004 and 2005, since when it has been in a short term ley with clover.

Evaluation of chemical, biological and nutritional treatments for overcoming replant disease in Sorbus (Experiment 3)

A field experiment was established in autumn 2009 in the two areas shown to differ in *Sorbus* replant disease severity (see Experiment 2 above). Treatments were done on the low risk site as well as the high risk site in order to help distinguish growth responses due to control of *Sorbus* replant disease from growth responses due to other factors.

Seven treatments were compared with an untreated control. Three soil disinfestation treatments were applied in autumn 2009: 'Custo-Fume' (98% chloropicrin) at 280 L/ha; 'Basamid' granules at 57 g/m^2; and 'Biofence' Caliente mustard meal (*Brassica carinata*) at 250 g/m^2. Soil temperature was around 11ºC and soil moisture was 17-23% at the time of these soil treatment applications. Treated areas were covered with clear polythene for 3

weeks (Custo-Fume) or 6 weeks. 'Novozyme Roots' (a mycorrhizal inoculant), 'PlantMate' granules and WP (*Trichoderma harzianum*) and 'Agralan Revive' (*Bacillus subtilis*) and one nutritional treatment (66.5 N kg/ha extra N, as $Ca(NO_3)_2$) were applied at and after planting *Sorbus aucuparia* in spring 2010. The soils were tested for free-living nematodes in September 2009 and very low numbers of stunt/spiral and root lesion nematodes were found, levels considered unlikely to affect crop growth.

'Custo-Fume' was the only treatment that significantly increased *Sorbus* growth on the high risk site. In the absence of a replant disease problem (the low risk site), none of the treatments significantly affected growth (Table 3).

Table 3: Effect of soil treatments on growth of *Sorbus aucuparia* in soils at high and low risk of replant disease – Tenbury Wells, 2010

Treatment	CRD approval status as a pesticide treatment (August 2011)	Active ingredient	Mean fresh weight per plant of top growth (g)[a]	
			High risk site	Low risk site
1. Untreated	-	-	51	166
2. Custo-Fume[b]	Label approval	Chloropicrin[f]	218	223
3. Basamid	Label approval	Dazomet	54	153
4. Biofence pellets	Not applicable[c]	mustard meal	40	136
5. Novozyme Roots	Exempt	Mycorrhiza	47	146
6. PlantMate	Experimental[d]	*Trichoderma harzianum*	84	148
7. Agralan Revive	Experimental[e]	*Bacillus subtilis*	82	169
8. Extra N	Not applicable[c]	350 kg $Ca(NO_3)_2$	49	131

[a] Includes weight of leaves. [b] Replicate plots were not randomised. [c]Fertiliser products, out of scope of pesticides legislation. [d]Used under an Experimental Permit. [e]Out of scope of pesticides legislation at the time it was used in this project. [f]Approval of this active is currently under review.

The failure of 'Basamid' to control replant disease under the conditions in this test was unexpected as there is evidence that it can provide control of replant disease in *Malus*. The soil conditions, including moisture and temperature, were favourable for both 'Basamid' and 'Custo-Fume'.

Graft bud failure was greater on untreated soil at the high risk replant disease site (35%) than the low risk site (7.5%). 'Custo-Fume' significantly reduced graft bud failure (0%).

Figure 1. Effect of 'Custo-Fume' (foreground left) on *Sorbus aucuparia* growth on the high risk replant disease site, August 2010

The control of *Sorbus* replant disease by 'Custo-Fume, a broad-spectrum soil fumigant, indicates that the cause may be of biological in origin. *Fusarium* spp. and *Pythium* spp. were recovered more frequently from roots of *Sorbus* grown in untreated soil than 'Custo-Fume' treated soil, suggesting these pathogens may be causal agents of Sorbus replant disease. A DNA test for 50 fungi in the two soils found large differences in the infestation levels of *Pythium* sp., with very high levels in untreated soil. *Pythium sylvaticum*, the predominant *Pythium* sp. associated with apple replant disease was not detected by the DNA test.

Financial benefits

Annual losses of marketable output of rosaceous trees in the UK to replant diseases are estimated to be around £1 million. The work in this experiment clearly showed the value of 'Custo-Fume' treatment both in increasing plant growth and reducing graft bud failure and the ineffectiveness of alternative treatments against this issue.

Action points for growers

- Based on the results obtained in this project, 'Custo-Fume' (chloropicrin) is the most effective pre-plant soil treatment for control of *Sorbus* replant disease. Following soil treatment with 'Custo-Fume' at 280 L/ha, weight of top growth on a high replant risk site was increased fourfold, from 51 g to 218 g.

- ‘Custo-Fume’ also reduced graft bud failure on trees affected by replant disease.
- ‘Basamid’, ‘Biofence’ seed meal, ‘Novozymes Roots’, ‘PlantMate’ granules / WP, ‘Agralan Revive’ and supplementary calcium nitrate as used in this project are unlikely to improve growth of *Sorbus aucuparia* trees on soils with a high risk or a low risk of *Sorbus* replant disease.
- A *Sorbus aucuparia* seedling bioassay to determine the occurrence of *Sorbus* replant disease in a soil generally gives a good indication of the risk but is not sufficiently reliable to offer as a commercial service.
- There is evidence from this project that *Pythium* spp. are a major component of the cause of Sorbus replant disease. Treatments to control pathogenic *Pythium* species at or soon after planting *Sorbus* warrant further investigation.

Science Section

Introduction

Previous work on replant diseases of rose and ornamental rosaceous trees is summarised in HNS 152 (O'Neill *et al*., 2007), which includes a full list of references. Most studies have been on apple replant disease, which is widespread and occurs in all the major apple production regions of the world. Replant diseases of ornamental rosaceous trees have been little studied. There appears to have been no work in the UK since that of Sewell at East Malling in the 1980s. Briefly, replant diseases of ornamental rosaceous trees are long-known and complex soil-borne problems. Fungi and actinomycete bacteria appear to be the most likely causes. There is evidence that even on a single host species more than one microorganism may be involved and the causes may differ between sites. Where trees are grown intensively, the disease is reported generally to affect the whole area.

The overall aim of the project is to reduce losses to replant diseases in rosaceous trees through the use of control methods other than synthetic broad-spectrum soil biocides. The specific objectives of the project are to:

1. Develop a *Sorbus* seedling bioassay in order to assess soils for the risk of replant disease when this species is grown. *Sorbus* has been selected for use in the bioassay as a highly susceptible indicator of replant disease.
2. Determine the efficacy of some biological and nutritional treatments in overcoming replant disease in *Sorbus*.

In year 1 we developed a *Sorbus* seedling bioassay. This involves measuring the increase in height over 12 weeks of *Sorbus aucuparia* seedlings in untreated soil and in the same soil that has been chloropicrin-treated, and calculating the growth response (R).

In year 2, bioassay methodology developed under objective 1 in year 1 was evaluated in two experiments. The first experiment was done to determine whether the bioassay could be used to differentiate soils with different predicted replant risk (based on known cropping histories). The second bioassay was used to test soils from prospective field sites for an experiment in 2010, to select areas of low and high replant risk. In addition, preparation for an experiment under objective 2 commenced in autumn 2009, with pre-planting treatments applied to plots that were planted with *Sorbus* in 2010.

The objectives in year 3 were:

1. To evaluate seven treatments for control of *Sorbus* replant disease on sites assessed as being at high risk and low risk of *Sorbus* replant disease;
2. To determine the correspondence of growth response of *Sorbus aucuparia* to chloropicrin soil treatment in pot tests and in the field.
3. To examine the effect of some pre-plant soil treatments and a fungicide drench on growth of *Malus* trees.

1. Evaluation of chemical, biological and nutritional treatments for overcoming replant disease in *Sorbus*

Introduction

A field trial commenced in 2009 with the objectives to:

- Determine the effect of two chemical soil disinfestation treatments, four biological treatments, and a nutritional treatment on growth of *Sorbus aucuparia*;
- Investigate the possible biological nature of *Sorbus* replant disease through the use of broad-spectrum soil disinfestation treatments;
- Determine the effect of treatments on *Sorbus* replant disease by comparison of the growth response in *Sorbus* on land with a high risk and a low risk of the factors causing *Sorbus* replant disease, based on cropping history on the two sites and bioassay results.

Methods

Site details

The experiment was sited at a farm in Worcestershire (F P Matthews Ltd, Tenbury Wells). The experiment comprised two field areas representing 'low risk' and 'high risk' areas for *Sorbus* replant disease, selected based on the results of bioassay experiments. The 'low risk' area was a grass headland / access route which has been uncropped for more than five years. The 'high risk' area was planted with lined out rootstocks in the spring of 2003, for budding that summer. A maiden crop of *Sorbus* and *Malus* was then grown during 2004, with a few trees then left in as a two year crop in 2005. Since 2005 it has been in a short term mixed ley with clover.

Soil treatments

The following chemical treatments were applied to both the 'high risk' and 'low risk' replant disease areas.

	Treatment	Application rate details
1.	Untreated control	-
2.	Custo-Fume (98% chloropicrin)	Injected to 20 cm depth pre-planting at 280 mL/m^2 (280 L/ha) and sealed in with polythene
3.	Basamid (98% dazomet)	Incorporated to 20 cm depth at 57 g/m^2 and sealed in with polythene
4.	'Biofence' Caliente mustard meal (*Brassica carinata*) pellets	Incorporated to 20 cm depth at 250 g/m^2 and sealed in with polythene.
5.	Novozymes 'MycorrhizaRoots'	Mix 200 g product in 100 L water, and apply at 1 L per plant, ensuring that the product infiltrates the rooting zone and does not run-off. Apply at planting at 8 weeks after planting .
6.	PlantMate granular root zone starter granules / PlantMate WP (*Trichoderma harzianum*)	Apply PlantMate granules to the planting hole (25 g) at planting then drench with PlantMate WP (1.5 kg/ha) at 4 and 8 weeks after planting.
7.	Agralan Revive (*Bacillus subtilis*)	Use as a root dip at 100 mL product in 10 L water (at planting), then drench to base of plants (100 mL in 10 L) at 4 and 8 weeks after planting
8.	Supplementary nitrogen	214.5 kg calcium nitrate (15.5% N) at 4 and 8 weeks after planting (429 kg in total) broadcast over whole plot

Treatments 2 and 3 are chemical soil disinfestation treatments. Treatments 4 to 7 are biological treatments. Treatment 8 was a nutritional treatment.

Treatments 2 to 4 were applied pre-planting in autumn 2009 and the remaining treatments were applied post-planting in spring 2010.

Experiment design

For each field area, the experiment comprised the eight treatments with four replicates arranged in randomised blocks; there was restricted randomisation of 'Custo-Fume' due to practical difficulties in application of this treatment to small plots. The dimensions and design of the low and high risk areas varied because of site limitations in terms of previous cropping history and available land (see trial plan in Appendix 1). Individual plots measured 2.75 m x 1 m and were planted with a single row of 10 one-year-old *S. aucuparia* seedlings spaced at 25 cm in April 2010. Unplanted paths were left across the ends of plots and between plots. The plants were budded with *Sorbus* 'Joseph Rock' in August 2010.

Land-preparation of the trial areas was done in September 2009 by the host grower, in advance of soil treatments with 'Custo-Fume', 'Basamid' and 'Biofence', with the aim of obtaining well cultivated soil with moisture throughout the soil profile.

'Custo-Fume' was applied to relevant plots on 1 October 2009 by Custodian Fumigation Ltd at a rate of 280 mL/m^2 (280 L/ha) to a depth of 20 cm depth, and covered with clear polythene sheeting (approximately 3.5 m width) that was spaded-in at the edges. The sheeting was removed after 3 weeks, following wind damage.

'Basamid' was applied at 57 g/m^2 and 'Biofence' at 250 g/m^2, both on the 8 October 2009. Both treatments were incorporated to 20 cm depth using a spading machine and plots were covered with clear polythene film that was spaded in at the edges. Plastic was removed from the 'Basamid' and 'Biofence' plots 6 weeks after treatment.

The remaining treatments were applied in spring 2010 at and after planting the *Sorbus* plants (see Appendix 3, trial diary, for details). One year-old *S. aucuparia* seedlings (5-7 mm) were planted on 27 April 2010.

Measurements and assessments

Soil from each trial area was sampled on 1 October 2009 and sent for analysis of pH, P, K, Mg, organic matter and micronutrients (NRM laboratories). Soil was again sampled on 16 March 2010 and sent for analysis of pH, P, K, Mg, soil mineral nitrogen (SMN), anaerobic mineralisable nitrogen (AMN) and soil textural classification (NRM laboratories). Soil samples collected in October 2009 were also sent for extraction of free-living nematodes (ADAS Pest Evaluation Services). Nematodes such as root lesion nematodes (e.g. *Pratylenchus penetrans*) have previously been implicated as contributing to replant disease. Base-line soil sampling for nematodes was done to determine whether later assessments were needed to determine i) levels of nematodes after treatment, and ii) root infestation levels.

Soil temperature (20 cm depth) and % soil moisture was determined at each application time. Monthly rainfall records were obtained from the host grower (Appendix 2).

Plants were assessed for vigour on a 1-5 scale (with 5 as most vigorous) on 2 July and 2 August 2010. The plants were budded with Joseph Rock on *S. aucuparia* on 12 August 2010. The tops were cut off just above the bud (15 cm above ground level, 5 cm above the bud) on 12 October 2010, as leaves were beginning to yellow, and weighed. Results were examined by analysis of variance.

In January 2011, trees were assessed for failure of grafted buds. The intention had been to measure extension growth in autumn 2011, but the severe winter weather of 2010/11 badly affected the young trees and this had to be abandoned.

In March 2011, five trees were forked up from each of the 'Untreated' and 'Custo-Fume' treated plots at the high risk site. After washing in tap water, 10 sections of fine root, around 5-10 mm in length from each tree, were surface sterilised in sodium hypochlorite (1% for 1 min), and plated onto a general growth medium (PDA+S), and one selective for Pythiaceous fungi (P_5ARP). The culture plates were assessed after 7 and 14 days (incubation at 20^{o}C), to determine the relative frequency of recovery of different fungi.

Also in March 2011, soil samples were collected from around roots of trees in the 'Untreated' and 'Custo-Fume' treated areas in the high risk site and sent to Scientia Terrae, Belgium, for a DNA multiscan test.

In April 2011, five representative trees from the 'Untreated' and 'Custo-Fume' treated plots at the high risk site were carefully forked up, the roots were washed free of soil, and root fresh weight was determined.

Trial maintenance

The experimental areas were managed by the host grower according to commercial practice. Fertiliser (11:4.5:18) was applied at 300 kg/ha across the low and high risk areas prior to planting. Details of pesticide treatments applied for control of foliar pests and diseases, herbicides for weed control, and irrigation applications, are given in Appendix 3.

Results and discussion

Soil treatments

At the time of 'Custo-Fume' application, soil temperature was 11.5ºC; percentage moisture was 16.8% for the high risk area and 15.0% for the low risk area. For the 'Basamid' and 'Biofence' treatments, soil temperature was 11ºC; percentage moisture was 22.8% for the high risk area and 22.1% for the low risk area.

The soil temperatures were satisfactory for effective treatment with 'Custo-Fume' (above 10ºC preferred) and 'Basamid' (above 7ºC preferred) and was likely to have been suitable for 'Biofence'.

'Basamid' requires a moist soil for the active ingredient (dazomet) to react with water and generate methyl–isothiocyanate (MITC), the active fumigant gas. In descriptive terms the soil should be moist yet still suitable to work on with tractor and machinery (P Shakespeare, Certis, pers. comm.). If a soil is too dry, 'Basamid' is very slow to breakdown and release MITC; if it is too wet there is poor diffusion by MITC through the soil. The ideal soil moisture

content for ‘Basamid’ treatment is recommended as a field capacity value, rather than a % soil moisture value; the preferred range for rapid release to give a high concentration of MITC in the soil after 24 h is 60-70% field capacity. There is no simple formula to convert from % soil moisture to % field capacity. One study giving both measures was found; for a sandy loam soil with a humus content of 1-2%, a field capacity of 60-70% equated to a moisture content of 20% (Neumann *et al*., 1984). The soil moisture at application in our work, to a clay loam soil, was 22.1% and 22.8%. Overall, it is considered likely that it was suitable for effective ‘Basamid’ treatment.

The precise moisture requirements for optimum results with ‘Custo-Fume’ and ‘Biofence’ are not specified. ‘Custo-Fume’ worked well (see below), so a moisture content of 15-17% is evidently satisfactory. With ‘Biofence’ the active fumigant is primarily a mixture of fungitoxic methyl–isothiocyanates liberated from glucosinolates by an enzymatic process; a high moisture level and warm conditions are likely necessary for optimum release with this process. Although the ‘Biofence’ treatment was not irrigated after incorporation, the material was present in warm, moist soil under polythene from 8 October 2009, with exposure to precipitation from 19 November 2009 until planting on 27 April 2010

Soil analyses

Results from soil analyses in October 2009 and March 2010 are summarised in Table 1.1 with the main feature being that available phosphate levels are high for both trial areas. Numbers of free-living nematodes in the trial areas are shown in Table 1.2. Interpretation of this data is that the low numbers of nematodes in these samples are unlikely to have a significant impact on most crops (S. Ellis, ADAS entomologist, pers. comm.).

Effect of treatments on top growth

At the crop vigour assessment on 2 August 2010, growth of plants in most treatments was much better on the low risk replant disease site than the high risk site (Table 1.3). On both sites, ‘Custo-Fume’ resulted in significantly improved growth compared with the untreated. None of the treatments reduced growth compared with the untreated.

Table 1.1: Analysis and interpretation of soil factors for *Sorbus* trial areas with differing replant risk, Worcestershire, October 2009 and March 2010

Analysis	High risk trial area			Low risk trial area		
	Oct	Mar		Oct	Mar	
pH	6.7	6.9		6.6	7.0	
Phosphorous Index	4	5		4	4	
Potassium Index	3	3		3	3	
Magnesium Index	3	3		4	4	
Phosphorous mg/L (available)	64.8	79.0	Good P status	58.2	62.6	Good P status
Potassium mg/L (available)	290	264		298	264	
Magnesium mg/L (available)	164	168		185	192	
Copper (mg/L)	7.5	-	Slightly high	7.1	-	Slightly high
Boron (mg/L)	1.3	-	Normal	1.1	-	Slightly low
Zinc (mg/L)	8.0	-	Slightly high	8.0	-	Slightly high
Iron (mg/L)	80.7	-	Normal	109.8	-	Normal
% Organic matter	2.9	-	Slightly low	2.7	-	Normal
Sulphate (mg/L)	28.9	-	Slightly low	36.2	-	Slightly low
Manganese (mg/L)	17.1	-	Normal	26.1	-	Normal
Textural classification			Clay			Clay
SMN (0-30 cm) (available N)		45.5 kg N/ha			56.5 kg N/ha	
SMN (30-60 cm) (available N)		42.0 kg N/ha			36.4 kg N/ha	
AMN (0-30 cm) (potential N)		85.0 kg N/ha			70.0 kg N/ha	

Table 1.2: Quantification of free-living nematodes in *Sorbus* trial areas, Worcestershire, October 2009

Area	Nematode numbers / litre soil					
	Stubby root	Stunt / spiral	Cyst juveniles	Root lesion	Needle	Dagger
High Risk	0	175	0	0	0	0
Low risk	0	575	0	300	0	0

Table 1.3: Effect of some fumigant, biological and nutritional treatments on *Sorbus* replant disease at two sites – 2010

Treatment	Crop vigour (1-5) on 2 August	
	Low risk site	High risk site
1. Untreated	3.8	3.1
2. Custo-Fume	**4.5**	**4.6**
3. Basamid	3.5	3.0
4. Biofence	3.8	2.9
5. Mycorrhiza Roots	3.9	2.9
6. PlantMate	3.7	3.2
7. Agralan Revive	4.0	3.2
8. Supplementary N	3.5	2.9
Significance (21 df)	0.049	<0.001
LSD	0.61	0.51

Treatments that differ significantly from the untreated are shown in bold.

Weight of top growth was determined in October 2010. This was considered to be a better measure of growth response than plant height as the number of leaves and branches differs between plants.

At the high risk site only 'Custo-Fume' significantly increased top growth (Table 1.4); 'PlantMate' and 'Agralan Revive' appeared to increase growth, but the difference from the untreated was not statistically significant (Table 1.4). At the low risk site, 'Custo-Fume' appeared to increase growth (P=0.056) but none of the other treatments did. Top growth of plants on 'Custo-Fume' treated soil was similar in the high and low risk soils.

Surprisingly, 'Basamid' in this work gave no growth stimulus in either of the soils. Work in France has shown that 'Basamid' soil treatment can increase growth and yield of apple trees on replanted site (Otto & Winkler, 1993) and this was also observed by the host grower for *Malus* (see Section 3). Possibly in our work, under the soil conditions at application, fumigation was more effective with 'Custo-Fume' than 'Basamid' even though temperature and moisture appeared suitable for both. Other possibilities are that application of 'Basamid' to relatively small plots (2.75 m^2) using the spading machine did not enable optimum results with the fumigant; or that the soil had previously been treated with 'Basamid' or similar fumigant and that there was accelerated degradation of dazomet, resulting in insufficient accumulation of the active gases in the soil. Grower experience at this site indicates that chloropicrin treatment, such as 'Custo-Fume', is generally the most effective soil fumigant for replant disease (supported by observations in *Malus* – see Section 3).

Table 1.4: Effect of soil treatments on growth of *Sorbus* in soils at high and low risk of replant disease – Tenbury Wells, 2010

Treatment	Active ingredient	Mean fresh weight per plant of top growth (g)[a]	
		High risk site	Low risk site
1. Untreated	-	51	166
2. Custo-Fume	**chloropicrin[b]**	**218**	223
3. Basamid	Dazomet	54	153
4. Biofence	Mustard meal	40	136
5. Novozyme Roots	Mycorrhiza	47	146
6. PlantMate WP	*Trichoderma harzianum*	84	148
7. Agralan Revive	*Bacillus subtilis*	82	169
8. Extra N	350 kg $Ca(NO_3)_2$	49	131
Significance (21 df)		<0.001	0.056
LSD		44.5	54.9

[a] Includes weight of leaves. [b] Replicate plots were not randomised.

Treatments that differ significantly from the untreated are shown in bold.

On both sites 'Biofence' appeared to reduce growth (this was also observed on *Malus*, see Section 3), although this was not statistically significant. A possible explanation is that 'Biofence' stimulated resident soil populations of pathogenic *Pythium* and *Phytophthora* spp., an effect reported with a brassica seed meal in Washington State, USA (Mazzola & Brown, 2010).

An alternative explanation for this effect on growth is that breakdown and release of active substances (isothiocyanates) from the seed meal occurs over a prolonged period, and/or the active substances persist in the soil for a long time. It is known that related products (methyl–isothiocyanate from 'Metam Sodium' and dazomet from 'Basamid') can result in stunted growth if a crop is planted too soon after treatment. In our work, both 'Basamid' and the 'Biofence' were applied in autumn 2009 and the trees were not planted until April 2010. Polythene sheets were removed 6 weeks after treatment but the soils were only cultivated on the day of planting (following commercial practice), so retention of isothiocyanates in the soil could have occurred; if so, this could explain the stunted growth observed with this treatment.

The lack of a growth response to the mycorrhizal treatment, 'Novozyme Roots', probably indicates that the product used in this work does not have an effect on *Sorbus* replant

disease, either through increasing host resistance or by other mechanisms, to the plant pathogens that cause the disease. Mycorrhizae can improve plant growth through increasing the availability of phosphorus on soils low in this element. The high available phosphorus found at both sites explains why the mychorrizal treatment failed to improve tree growth through a nutritional effect.

There was a trend towards a growth response with both 'PlantMate' (*Trichderma harzianum*) and 'Agralan Revive' (*Bacillus subtilis*) at the high risk site (Table 1.4) but in neither case was the difference statistically significant. Possibly different strains of these micro-organisms might be effective; additionally, it may be worth testing a combination of these treatments.

The extra nitrogen had no effect on plant growth on this site where soil mineral nitrogen (45-60 kg/ha) was in adequate supply. This result indicates that extra nitrogen is unlikely to allieviate *Sorbus* replant disease on sites where soil mineral nitrogen is readily available.

There was a significant difference between blocks ($P < 0.001$) in the low risk soil, but not in the high risk soil ($P = 0.932$), indicating that a factor other than replant disease was affecting growth, or the cause of replant disease was present but not uniformly distributed in soil at the low risk site. Foliar weights were significantly lower for blocks 1 and 3, and examination of plot data shows that this was largely due to lower plot weights, irrespective of treatment at one end of the trial area (Appendix 4). This may have been due to the trial extending at one end of the low risk plots into an area that had been cultivated more recently with *Sorbus*, such that replant risk was higher at one end of the low risk trial area (N. Dunn, pers. comm.).

Effect of treatment on bud take

At the high risk site, treatment had a significant effect on the failure of buds to graft (Table 1.5). Bud failure was least in 'Custo-Fume' treated trees (0 out of 10) compared with 3.5 out of 10 in the untreated plots. The 'Basamid', 'PlantMate' and supplementary N treatments all appeared to reduce bud failure, while 'Biofence' appeared to increase it (5.25 out of 10), but this was not a statistically significant effect.. At the low risk site, bud failure was low in all treatments (0.25-0.75 out of 10) with no significant difference between treatments.

Effect of treatment on root growth

When plants were forked up in March 2011, there was a visibly greater extent of fine roots, and a greater thickness to medium roots in trees grown in 'Custo-Fume' treated soil compared with those in untreated soil (Fig. 1.2). In April 2011, when the roots from five trees were weighed, the increase in both root weight and top growth weight following

'Custo-Fume' treatment was around fourfold (Table 1.6). Root weight of trees from untreated plots ranged from 19.3 to 29.1 g; that from 'Custo-Fume' treated plots ranged from 57.4 to 129.1 g. There was no obvious rotting of roots, either on trees from untreated or 'Custo-Fume' treated soils.

Table 1.5: Effect of soil treatments on bud take of *Sorbus* in soils at high and low risk of replant disease – Tenbury Wells, 2011

Treatment	Active ingredient	Mean number buds failed (of 10)	
		High risk site	Low risk site
1. Untreated	-	3.50	0.75
2. Custo-Fume	**Chloropicrin[a]**	**0.0**	0.75
3. Basamid	Dazomet	1.50	0.50
4. Biofence	mustard meal	5.25	0.25
5. Novozyme Roots	Mycorrhiza	3.00	0.25
6. PlantMate WP	*Trichoderma harzianum*	1.50	0.50
7. Agralan Revive	*Bacillus subtilis*	2.00	0.25
8. Extra N	350 kg $Ca(NO_3)_2$	1.75	0.75
Significance (21 df)		0.040	NS
LSD		2.868	-

[a] Replicate plots were not randomized.

Table 1.6: Effect of soil treatments on fresh weight of top growth and roots of *Sorbus* in soils at high risk of replant disease – Tenbury Wells, 2010 and 2011

Treatment	Active ingredient	Mean fresh weight per plant (g)	
		Top growth[a] (October 2010)	Root growth[b] (April 2011)
1. Untreated	-	51	23.4
2. Custo-Fume	**chloropicrin[b]**	**218**	84.1
3. Basamid	Dazomet	54	-
4. Biofence	mustard meal	40	-
5. Novozyme Roots	Mycorrhiza	47	-
6. PlantMate WP	*Trichoderma harzianum*	84	-
7. Agralan Revive	*Bacillus subtilis*	82	-
8. Extra N	350 kg $Ca(NO_3)_2$	49	-

[a]Mean of 10 plants per plot; [b]mean of 5 plants from across replicate plots of treatments 1 and 2; other treatments were not examined.

Figure 1.2. Comparison of growth after 1 year; note the thicker stem bases and greater root mass in trees from 'Custo-Fume' treated.

Recovery of fungi from roots

Pre-plant soil treatment with 'Custo-Fume' significantly reduced root infection by species of *Cylindrocarpon, Fusarium* and *Pythium*, all potential pathogens of *Sorbus* roots (Table 1.7 and 1.8). The predominant fungal groups were *Fusarium* and *Pythium*, and 'Custo-Fum'e reduced both by around 75%. Occurrence of *Pythium* species was greater on medium thickness than fine roots, whereas *Fusarium* occurrence was unaffected by root thickness.

The DNA multiscan test developed by Scientia Terrae, Belgium (www.scientiaterrae.org), simultaneously examines a sample for unique DNA fragments of around 50 plant pathogenic fungi and oomycetes and some beneficial fungi. Levels of fungi and oomycetes detected are quantified on a 0-3 scale where 0 = not detectable, 1 = light infestation, 2 = moderate infestation and 3 = high infestation. The DNA multiscan test detected *Cylindrocarpon destructans, Fusarium* sp., *Fusarium oxysporum* and *Trichoderma* sp. closely matching the results obtained by isolation from roots onto agar. *Pythium* sp. was detected at a high infestation level in untreated soil and was not detected in Custo-Fume treated soil (Table 1.9 and Appendix 7).

The DNA test did not detect *P. sylvaticum* or *P. ultimum*, both of which have previously been associated with apple replant disease. Possibly this indicates these species are not involved as causes of *Sorbus* replant disease. Alternatively, these species may be present

(and classed as *Pythium* sp. by the test), and the DNA test did not detect the particular isolates present in our soil.

Pythium species are reported to be the likely cause of replant disease in apple, notably *P. sylvaticum* (UK), *P. indigoferae* and *P. irregulare* (Tunisia) and *P. ultimum* and *P. sylvaticum* (Washington State, USA) (Sewell, 1981; Souli *et al*., 2011; Mazzola, 1998). Grower observations indicate that *Sorbus* replant disease can occur in *Sorbus* planted after only *Malus*. The results of our isolations from roots, the soil DNA multiscan test and the grower observation, taken together, indicate that *Pythium* spp. are a likely major component of the cause of *Sorbus* replant disease. It is recommended that future work on control of *Sorbus* replant disease should focus on treatments aimed at control of *Pythium* root rot.

Interestingly, *Trichoderma* sp. was recovered only from roots of trees grown in 'Custo-Fume' treated soil. Increases in soil population of *Trichoderma* species after chloropicrin treatment, which can remain elevated for several months, have been noted previously (South *et al*., 1997). Potentially these may be beneficial by delaying re-colonisation of roots in fumigated soil by fungal pathogens.

Table 1.7: Effect of pre-plant soil treatment with 'Custo-Fume' and root thickness on recovery of fungi and bacteria from roots of *Sorbus aucuparia* grown for 10 months on a site with *Sorbus* replant disease

Treatment	Root size	No. root pieces (of 100 per size) affected by:			
Potential pathogens		*Colletotrichum*	*Cylindrocarpon*	*Fusarium*	*Pythium*
Untreated	Fine	1	3	39	5
	Medium	0	1	46	14
	Total	1	4	85	19
Custo-Fume	Fine	2	0	11	1
	Medium	0	0	8	4
	Total	2	0	19	5
Saprophytes		*Mucor*	*Trichoderma*	Unidentified	Bacteria
Untreated	Fine	21	0	28	13
	Medium	35	0	18	6
	Total	56	0	46	19
Custo-Fume	Fine	35	5	18	5
	Medium	50	4	7	0
	Total	85	9	25	5

Table 1.8: Effect of pre-plant soil treatment and root thickness on fungi and bacteria recovered from roots of *Sorbus aucuparia* grown on a site with *Sorbus* replant disease – Significance values

Micro-organism	F probability values		
	Soil treatment	Root thickness	Soil treatment x Root thickness
Collectotrichum	0.037	<0.001	NS
Cylindrocarpon	<0.001	<0.001	NS
Fusarium	<0.001	NS	NS
Pythium	<0.001	<0.001	NS
Mucor	0.013	0.007	NS
Trichoderma	<0.001	NS	NS
Unidentified fungi	0.018	<0.001	NS
Bacteria	<0.001	<0.001	0.003

Table 1.9: Infestation levels of fungi and oomycetes in soil from around roots of *Sorbus* trees severely affected by replant disease and unaffected trees ('Custo-Fume' treated pre-planting) as determined by a DNA multiscan test.

Fungus	Infestation level (0-3) in soil from:	
	Untreated	Custo-Fume treated
Cylindrocarpon destructans	1	1
Fusarium sp.	2	3
Fusarium oxysporum	1	1
Pythium sp.	3	0
Trichoderma sp.	1	3

Full results are detailed in Appendix 7.

2. Comparison of *Sorbus* growth response to chloropicrin in pots and in the field

Introduction

The aim of this experiment was to use a *Sorbus* seedling assay developed in Year 1 to determine replant risk in two soils and to compare the response to 'Custo-Fume' (chloropicrin) treatment in the same soils in the field.

Methods

Soil source

Soil was sourced in April 2010 from four of the treatments at the site of the replant disease control experiment described in Section 1. Forty litres of soil was collected from each treatment.

Table 2.1: Details of soils collected from selected treatments in two field areas differing in replant disease risk

Soil source		Soil treatment in field
1	Low risk replant disease area	Untreated
2	Low risk replant disease area	Custo-Fume (chloropicrin 280 L/ha)
3	High risk replant disease area	Untreated
4	High risk replant disease area	Custo-Fume (chloropicrin 280 L/ha)

Soil disinfestation

Each of the four soil source types was mixed thoroughly by tumbling the bags, which were then divided into two equal samples (approx 20 L each). One sample received no treatment (Untreated). The second sample received 'Custo-Fume' (chloropicrin (CP)) treatment at Custodian Fumigation (Eye, Suffolk). Treatment by Custodian Fumigation was as follows: soil was packed into polythene-lined boxes and chemical applied as if this was a seed bed within a field. Following fumigation with 'Custo-Fume' at 280 L/ha, the soil was polythene covered. Temperature was 18ºC during treatment. Venting commenced 14 days after fumigation, with soil turning and spreading. Soil % moistures are given in Table 2.2. A cress test was done with the fumigated soil samples, to ensure that fumes had dissipated prior to seedling transplanting.

Table 2.2: Details of soil % moisture prior to 'Custo-Fume' (chloropicrin (CP)) treatment - 2010

Soil Source	Collected from field treatment	Predicted replant severity	Moisture % of FC
1.	Low risk replant area – Untreated	Low	44.7
2.	Low risk replant area – CP	Low	40.8
3.	High risk replant area –Untreated	High	46.0
4.	High risk replant area – CP	Low	51.0

Seedling production

Stratified seeds of *Sorbus aucuparia* were obtained from Forestart Ltd, using the same provenance as for a previous bioassay experiment in 2008 (provenance UK origin, region 109 Scotland). The seeds were refrigerated on arrival and sown within 2 days of receipt, in F2+S compost in 100 half seed trays (20 seeds per tray) at 1 cm depth. The trays were placed on capillary matting in a glasshouse at approximately 20ºC and covered with fleece. Water was sprayed from a hand-held mister to keep the surface of the compost damp, rather than overhead watering. Emergence counts were done at two-weekly intervals after planting.

Experiment Design

The experiment comprised a randomised complete block design in a polytunnel with the eight soil disinfestations treatments replicated in four blocks. A plot comprised five 20 x 1 L pots with one 6 week old *Sorbus* seedling per pot, placed in a chitting tray on capillary matting, giving a total of 32 plots, and a total of 160 seedlings for the trial. A temperature logger (Tinytag) was placed in the polytunnel to record air temperature hourly.

Trial maintenance

Initially the pots were covered in fleece to aid seedling establishment. Fleece was removed after around 4 days. The soil and capillary matting was overhead-watered by hand to ensure it remained moist but not water-logged. From approximately 4 weeks after transplanting a feed containing phosphorous (Vitafeed 214, 1:100 dilution) was applied weekly at a rate of 100 ml per 1 L pot. *Encarsia* and *Amblyseius* biological control agents were applied fortnightly for control of whitefly and thrips, respectively. A trial diary is given in Appendix 6.

Growth assessments

For each plant, seedling vigour (0-5 scale) was assessed at 9 weeks after transplanting. Height was measured from the soil level to tip of the growing point at 1 week after transplanting (baseline height) and 12 weeks after transplanting.

Foliar and root weight assessments

After the last height assessment, the fresh weight of all plants to soil level was recorded. In addition, the root system was removed from 10 plants per treatment, washed in tap water, dried with paper towel then weighed. Washed roots from five plants per treatment were plated onto potato dextrose agar amended with streptomycin (five plants per treatment, one plate of four root pieces per plant). Plates were incubated at 20ºC, and were then checked for growth of different fungal species after 5 and 12 days.

Data analysis

Differences in growth at intervals after transplanting, plant fresh weights and root weights were analysed using ANOVA in Genstat. In addition, data were investigated using the methods described for the SARD test (Sewell *et al*., 1988) in which the percent growth response (R) of apple seedlings to soil fumigation was calculated from the formula: %R = 100 F/U where F and U respectively represented the mean actual heights 12 weeks after transplanting of plants in fumigated and unfumigated soil. For *Sorbus* data obtained in this experiment, %R was calculated separately using i) height differences at intervals after transplanting (week 9–week 1, and week 12–week 1), ii) root fresh weights at 12 weeks after transplanting, and iii) leaf/stem fresh weights at 12 weeks after transplanting.

Results and discussion

At the plant vigour assessment at 9 weeks after potting, seedlings grown in soil treated with 'Custo-Fume' at the pot stage were significantly more vigorous than seedlings grown in soil not treated at this stage (Table 2.3). There were also significant field treatment x pot test and soil source x field treatment x pot test interactions, suggesting greater vigour following field and pot treatment with 'Custo-Fume'.

At 12 weeks after transplanting, seedling height was unaffected by soil source (low or high risk replant disease areas), field treatment ('Custo-Fume' treated or not) or pot soil treatment ('Custo-Fume' or not) (Table 2.4). With seedling top weight there was a significant soil source x pot test interaction. This result was surprising with least weight in the low risk soil untreated with 'Custo-Fume' at the pot stage, and greatest weight in the high risk site untreated with 'Custo-Fume' at the pot stage (Table 2.5). With root weight, there was a highly significant effect from soil source (root weight was significantly less in the

high risk site soil than the low risk site soil), and pot soil treatment (greater root weight in soil treated with 'Custo-Fume' at the pot stage) (Table 2.6). Field 'Custo-Fume' treatment also appeared to increase root weight, though the effect was not quite significant (P = 0.092).

The growth response (R) based on top weight of *Sorbus* plants grown in the field broadly matched that predicted by the pot test used to select the low and high risk *Sorbus* replant disease sites (Table 2.7). However, when fresh samples of these soils were re-tested by the pot bioassay in 2010, none of the measures of R (increase in height, top weight, root weight) were similar to those found in the field (Table 2.8).

The reason for the bioassay in 2010 failing to reflect the growth achieved in the field is unclear. One possible explanation is that the soils collected in 2010 were not truly representative of the areas planted in the field. Soil samples were taken after the 'Custo-Fume' field treatment had been applied and were constrained by the need to minimise soil removal from the plots being planted. The untreated soils for both the low and high risk sites were a mixture of soils taken from the four untreated plots in each area and from untreated soil between treatment plots.

An alternative explanation is that the 'Custo-Fume' treatment applied to soils after removal from the field was not fully effective. The increases in seedling height and top weight (though not root weight) in the high risk site soils were relatively small following 'Custo-Fume' treatment of removed soils, compared with those observed in the field.

A third possible explanation is that soils collected from the same areas some months apart were different in microbial population composition or activity; and growth of *Sorbus* seedlings in these soils either untreated or 'Custo-Fume'-treated responded differently.

Table 2.3: Effect of soil source, field 'Custo-Fume' (chloropicrin) treatment and pot test 'Custo-Fume' (chloropicrin) treatment on growth of *Sorbus* seedlings vigour 9 weeks after transplanting – 2010

Factor	Vigour score (1 – 5)	Significance (21 df)	Reps	LSD
Soil source				
Low risk replant site	3.6	NS	21	-
High risk replant site	3.7			
Field treatment				
Untreated (F-Nil)	3.4	NS	16	-
Chloropicrin (F-CP)	3.9			
Pot test treatment				
Untreated (P-Nil)	3.0	<0.001	16	0.465
Chloropicrin (P-CP)	4.3			
Soil source x Field treatment				
Low risk x F-Nil	3.5	NS	8	-
Low risk x F-CP	3.8			
High risk x F-Nil	3.4			
High risk x F-CP	4.0			
Soil source x Pot test treatment				
Low risk x P-Nil	3.0	NS	8	-
Low risk x P-CP	4.3			
High risk x P-Nil	3.0			
High risk x P-CP	4.4			
Field treatment x Pot test				
F-Nil x P-Nil	2.5	0.02	8	0.658
F-Nil x P-CP	4.4			
F-CP x P-Nil	3.5			
F-CP x P-CP	4.3			
Soil source x Field x Pot test				
Low risk x F-Nil x P-Nil	2.3	0.006	4	0.930
Low risk x F-Nil x P-CP	4.8			
Low risk x F-CP x P-Nil	3.8			
Low risk x F-CP x P-CP	3.8			
High risk x F-Nil x P-Nil	2.8			
High risk x F-Nil x P-CP	4.0			
High risk x F-CP x P-Nil	3.3			
High risk x F-CP x P-CP	4.8			

Table 2.4: Effect of soil source, field 'Custo-Fume' (chloropicrin) treatment and pot test Custo-Fume' (chloropicrin) treatment on *Sorbus* seedling height 12 weeks after transplanting – 2010

Factor	Seedling height (cm)	Significance (21 df)	Reps	LSD
Soil source				
Low risk replant site	43.8	NS	21	-
High risk replant site	49.4			
Field treatment				
Untreated (F-Nil)	45.7	NS	16	-
Chloropicrin (F-CP)	47.4			
Pot test treatment				
Untreated (P-Nil)	45.3	NS	16	-
Chloropicrin (P-CP)	47.9			
Soil source x Field treatment				
Low risk x F-Nil	43.7	NS	8	-
Low risk x F-CP	43.9			
High risk x F-Nil	47.8			
High risk x F-CP	50.9			
Soil source x Pot test treatment				
Low risk x P-Nil	39.1	NS	8	-
Low risk x P-CP	48.5			
High risk x P-Nil	51.4			
High risk x P-CP	47.3			
Field treatment x Pot test				
F-Nil x P-Nil	44.7	NS	8	-
F-Nil x P-CP	46.8			
F-CP x P-Nil	45.9			
F-CP x P-CP	48.9			
Soil source x Field x Pot test				
Low risk x F-Nil x P-Nil	40.4	NS	4	-
Low risk x F-Nil x P-CP	46.9			
Low risk x F-CP x P-Nil	37.8			
Low risk x F-CP x P-CP	50.0			
High risk x F-Nil x P-Nil	48.9			
High risk x F-Nil x P-CP	46.8			
High risk x F-CP x P-Nil	54.0			
High risk x F-CP x P-CP	47.8			

Table 2.5: Effect of soil source, field 'Custo-Fume' (chloropicrin) treatment and pot test Custo-Fume' (chloropicrin) treatment on growth of *Sorbus* seedling top weight 12 weeks after transplanting – 2010

Factor	Seedling top weight (g)	Significance (21 df)	Reps	LSD
Soil source				
Low risk replant site	22.5	NS	21	-
High risk replant site	25.7			
Field treatment				
Untreated (F-Nil)	23.2	NS	16	-
Chloropicrin (F-CP)	25.0			
Pot test treatment				
Untreated (P-Nil)	23.4	NS	16	-
Chloropicrin (P-CP)	24.9			
Soil source x Field treatment				
Low risk x F-Nil	20.7	NS	8	-
Low risk x F-CP	24.4			
High risk x F-Nil	25.8			
High risk x F-CP	25.6			
Soil source x Pot test treatment				
Low risk x P-Nil	19.0	0.035	8	7.3
Low risk x P-CP	26.1			
High risk x P-Nil	27.7			
High risk x P-CP	23.7			
Field treatment x Pot test				
F-Nil x P-Nil	22.9	NS	8	-
F-Nil x P-CP	23.6			
F-CP x P-Nil	23.8			
F-CP x P-CP	26.2			
Soil source x Field x Pot test				
Low risk x F-Nil x P-Nil	19.7	NS	4	-
Low risk x F-Nil x P-CP	21.6			
Low risk x F-CP x P-Nil	18.2			
Low risk x F-CP x P-CP	30.5			
High risk x F-Nil x P-Nil	26.0			
High risk x F-Nil x P-CP	25.6			
High risk x F-CP x P-Nil	29.5			
High risk x F-CP x P-CP	21.8			

Table 2.6: Effect of soil source, field 'Custo-Fume' (chloropicrin) treatment and pot test Custo-Fume' (chloropicrin) treatment on growth of Sorbus seedling root weight 12 weeks after transplanting – 2010

Factor	Root weight (g)	Significance (21 df)	Reps	LSD
Soil source				
Low risk replant site	26.8	0.003	21	3.87
High risk replant site	20.7			
Field treatment				
Untreated (F-Nil)	22.1	NS	16	-
Chloropicrin (F-CP)	25.4			
Pot test treatment				
Untreated (P-Nil)	21.7	0.038	16	3.87
Chloropicrin (P-CP)	25.8			
Soil source x Field treatment				
Low risk x F-Nil	26.6	NS	8	-
Low risk x F-CP	26.9			
High risk x F-Nil	17.5			
High risk x F-CP	23.9			
Soil source x Pot test treatment				
Low risk x P-Nil	25.7	NS	8	-
Low risk x P-CP	27.8			
High risk x P-Nil	17.6			
High risk x P-CP	23.8			
Field treatment x Pot test				
F-Nil x P-Nil	18.9	NS	8	-
F-Nil x P-CP	25.2			
F-CP x P-Nil	24.4			
F-CP x P-CP	26.4			
Soil source x Field x Pot test				
Low risk x F-Nil x P-Nil	22.9	NS	4	-
Low risk x F-Nil x P-CP	30.4			
Low risk x F-CP x P-Nil	28.6			
Low risk x F-CP x P-CP	25.2			
High risk x F-Nil x P-Nil	15.1			
High risk x F-Nil x P-CP	20.0			
High risk x F-CP x P-Nil	20.2			
High risk x F-CP x P-CP	27.6			

Table 2.7: Growth response of *Sorbus* in two soils to 'Custo-Fume' (chloropicrin) treatment as measured by a pre-plant bioassay in 2009 and in the field in 2010.

Predicted risk of replant disease based on cropping history	Growth response (R) determined on:	
	2009	2010
	Seedling height, bioassay	Plant weight, in field
Low risk	174	134
High risk	304	427

A replant disease problem is considered to occur when the bioassay value R≥150, and a severe problem when R ≥200.

Table 2.8: Growth response of *Sorbus* in two soils to 'Custo-Fume' (chloropicrin) treatments as measured by a seedling bioassay in 2010 and in the field in 2010

Predicted risk of replant disease based on cropping history	Growth response (R) determined on:			
	Seedling height	Seedling top weight	Seedling root weight	Plant weight in field
Low risk	116	110	133	134
High risk	96	98	133	427

3. Effect of soil treatments and a fungicide drench on growth of *Malus* trees

Introduction

Recent work in the USA (Mazzola *et al*., 2009; Mazzola & Brown, 2010) found that a *Brassica juncea* seed meal pre-plant soil amendment, used in combination with a post-plant application of a fungicide containing the active ingredient metalaxyl-M, was as effective as chloropicrin in control of apple replant disease. The brassica seed meal on its own was ineffective as it stimulated resident populations of pathogenic *Pythium* spp. and *Phytophthora cambivora* in the soil that infected apple roots. Whilst working at the *Sorbus* trial site in this project, opportunity was taken to examine the effect of a single drench with 'Subdue' (metalaxyl-M), applied alone or in conjunction with 'Biofence' soil treatment (*Brassica carinata* seed meal), on growth of ornamental *Malus* trees.

Materials and methods

Site and crop details

The work was done on a field in Worcestershire with a history of replant disease in *Sorbus* and *Malus*. The previous crop on the field was grass/clover ley put up in the furrow for the winter. Large field strips (approx. 200 x 3 metres) had been treated with 'Custo-Fume' or 'Biofence' (managed by the host grower) or left untreated in autumn 2009. The field was planted with one-year-old *Malus* on 20 April 2010. Trees were planted 25 cm apart in the row and 92 cm between rows.

Treatments

There were five treatments:

1. Untreated
2. 'Custo-Fume' (98% chloropicrin) at 280 L/ha (autumn 2009)
3. 'Subdue' (465 g/L metalaxyl-M) at 1.25 L/ha (July 2010)
4. Biofence at 2,500 kg/ha (autumn 2009)
5. 'Biofence' at 2,500 kg/ha (autumn 2009) + Subdue at 1.25 L/ha (July 2010)

Subdue was applied on 9 July 2010 at the maximum permitted drench rate (SOLA 0722/07) for outdoor ornamentals of 1.25 L/ha around tree bases (around 12 weeks after planting). This was achieved using a spray volume of 3,000 L/ha, in a 50 cm wide band, at a concentration of 0.417 ml/L. As there was no rain after planting, irrigation at 25 mm was applied twice at approximately 10 days apart.

Experiment design

There were four replicate plots of each treatment arranged in a run or a square approximately 1 m apart. It was not possible to arrange treatments in randomised blocks as the pre-plant treatments ('Untreated', 'Custo-Fume' and 'Biofence') had been applied in single large strips, arranged by the host grower. Plot size was a run of 10 trees. Results were examined by analysis of variance.

Assessment

Crop vigour was assessed on a 1-5 scale (with 5 indicating greatest vigour) on 2 August 2010. Trees were cut off at the base in October, 5 cm above the bud, and the weight of top growth determined.

Results and discussion

At the interim assessment of crop vigour, the growth of plants on 'Custo-Fume' treated soil was significantly better than other treatments (Table 3.1). When weight of top growth was determined in October, neither 'Custo-Fume' nor 'Subdue' had a statistically significant effect on growth compared with the untreated; both appeared to increase growth slightly (by around 8%) (Table 3.2). 'Biofence' significantly reduced growth and this effect was not negated by Subdue in a 'Biofence'/'Subdue' combined treatment.

Table 3.1: Effect of some soil treatments and a fungicide drench on *Malus* replant disease at an interim assessment – 2010

Treatment[a]	Crop vigour (1-5) on 2 August
1. Untreated	3.4
2. Custo-Fume	**3.8**
3. Subdue	3.4
4. Biofence	3.2
5. Biofence + Subdue	3.3
Significance (15 df)	<0.001
LSD	0.21

[a] Treatments were replicated four times but plots were not randomised as the experiment was established after application of treatments to large strips in a field.

Treatments that differ significantly from the untreated are shown in bold.

Table 3.2: Effect of soil treatments and a fungicide drench on growth of *Malus* trees – Tenbury Wells, 2010

Treatment[a]	Mean fresh weight per plant of top growth (g)
1. Untreated	247
2. Custo-Fume	268
3. Biofence	**162**
4. Subdue[b]	267
5. Biofence + Subdue	**121**
Significance (15 df)	<0.001
LSD	30.6

[a] Treatments were replicated four times but plots were not randomised as the experiment was established after application of treatments to large strips in a field.

Treatments that differ significantly from the untreated are shown in bold.

These results provide some evidence that there was a *Malus* replant problem in the field as crop vigour was significantly increased by 'Custo-Fume'. Both 'Custo-Fume' and 'Subdue' applied on their own appeared to increase weight of top growth, compared with untreated, though differences were not statistically significant. The most notable effect was the significant reduction in weight of top growth on land treated with Biofence; a similar effect was observed with Biofence soil treatment and growth of *Sorbus* (see Section 2).

Corresponding results were obtained by the host grower as follows: in adjacent commercial strips of *Malus* treated pre-planting with either 'Custo-Fume', 'Basamid' or 'Biofence', there were visible differences in growth between treatments, with trees treated with 'Custo-Fume' showing excellent growth compared with the 'Basamid' treatment (moderate growth) and 'Biofence' treatment (poor growth).

Before firm conclusions are drawn as to the potential benefit of 'Subdue' treatments in control of replant disease, it is recommended that a randomised block trial is done, and that Subdue is applied within 1 week of planting, as used in the USA. The concentration and drench volume of metalaxyl-M used in the USA was not specified, but use of a higher volume drench may warrant examination to ensure effective root coverage.

Conclusions

Bioassay development - Year 1

- A *Sorbus aucuparia* seedling growth bioassay has been devised. The test successfully differentiated two soils considered to be at 'low risk' (no rosaceous crops for over 20 years) and 'high risk' (soils from around *Sorbus* trees) of *Sorbus* replant disease.
- 'Custo-Fume' is preferred over steaming to treat soil for use in a *Sorbus* seedling bioassay. Soil steaming (autoclave treatment) resulted in very large growth response and may falsely predict a *Sorbus* replant disease problem on 'low risk' soil.
- 'Custo-Fume' greatly increases root and shoot fresh weight of *Sorbus aucuparia* seedlings grown in pots of 'high risk' replant soil in a seedling bioassay.

Bioassay testing - Year 2

- Soils from different sources can generally be differentiated with regard to risk of *Sorbus* replant disease using the *Sorbus* seedling bioassay developed in year 1; the bioassay is based on increase in height of *Sorbus* seedlings grown in 'Custo-Fume'-treated soil compared with untreated soil. The measured *Sorbus* replant disease severity risk was generally, but not always, in accord with a predicted risk based on history of rosaceous crops and recent use of 'Custo-Fume' on the soil.
- A range of bacteria and fungi are associated with roots of *Sorbus aucuparia* seedlings grown in soils for 12 weeks, including *Cylindrocarpon* sp. *Fusarium* sp. and *Pythium* sp., fungi which have previously been reported associated with replant diseases in *Malus*.

Effect of treatments on *Sorbus* replant - Year 3

- Replant disease can have a large effect on growth of *Sorbus aucuparia* seedlings in their first year of growth. The fresh weight of one season's top growth was three times greater on land predicted to be at low risk of replant disease than on land just a few metres away predicted to be at high risk of *Sorbus* replant disease. There were no obvious differences in soil pH, texture, organic matter, major nutrients, or soil nematode levels, to account for this difference.
- 'Custo-fume' (chloropicrin) soil treatment can give very good control of replant disease. Following soil treatment with 'Custo-Fume' at 280 L/ha, weight of top growth on a high risk site was increased fourfold, from 51 g to 218 g.

- We found that ‘Basamid’ and ‘Biofence’ applied pre-planting and ‘Novozymes Roots’ (a mycorrhizal product), ‘PlantMate’ granules and WP (*Trichoderma harzianum*), ‘Agralan Revive’ (*Bacillus subtilis*) and supplementary nitrogen ($Ca(NO_3)_2$) applied at and/or post-planting, were ineffective at controlling *Sorbus* replant disease on a site at high risk of the disease.
- ‘Custo-Fume’ may increase growth of *Sorbus* on land at low risk of *Sorbus* replant disease. Following soil treatment with Custo-Fume at 280 L/ha, weight of top growth on a low risk site was increased from 166 g to 223 g.
- The growth response of *Sorbus* in two field locations to ‘Custo-Fume’ soil treatment, measured by increase in weight of top growth over 1 year, broadly agreed with that predicted by a 12-week pot bioassay, measured by increase in height. However, a repeat *Sorbus* seedling bioassay using fresh soil samples from the same two field locations as above, collected one year later, showed almost no difference between them as measured by increase in seedling height, weight of top growth or weight of roots.
- *Sorbus* replant disease can affect graft bud take. Graft bud failure was greater on a high risk replant disease site (3.5 out of 10) than the low risk site (0.75 out of 10). At the high risk site, ‘Custo-Fume’ significantly reduced graft bud failure to zero.
- There is evidence that ‘Custo-Fume’ controls replant disease in *Malus*. The vigour of *Malus* trees in August 2010 on land treated with ‘Custo-Fume’ the previous autumn was greater than that of trees on untreated land, or following ‘Biofence’ treatment.
- ‘Biofence’ (*Brassica carinata*) soil treatment as used in this work can have an adverse effect on growth of *Malus* and *Sorbus* seedlings. The reason for this effect is unknown. Elsewhere it has been reported that *Brassica juncea* seed meal incorporated into soil can stimulate *Pythium* and *Phytophthora* species.
- ‘Subdue’ (465 g/L metalaxyl-M) used as a single drenching spray around tree bases in July, around 12 weeks after planting, had no effect on growth of *Malus* when applied alone or in conjunction with ‘Biofence’.
- Control of *Sorbus* replant disease by Custo-Fume indicates the cause is biological in origin. There is evidence from this project (from conventional mycological techniques and molecular methods) that species of *Pythium*, and possibly also *Cylindrocarpon* and *Fusarium*, are causal agents of *Sorbus* replant disease.

Technology transfer

Presentation

Replant diseases of *Sorbus*. HTA/HDC Rose R & D Forum, NIAB, Cambridge, 4 December 2008 (Tim O'Neill).

Articles

O'Neill T & Green K (2008). Treatments for replant disease. *HDC News* **148**, p8.

O'Neill T & Green K (2009). A test for soil sickness. *HDC News* **158**, 28-29.

O'Neill T & Green K (2011). Sorbus replant treatments tested. *HDC News* (in press).

Project review meetings

17 March 2009, Worcs (T. O'Neill, K. Green, D. Churchill, N. Dunn, J. Adlam, T. Locke, J. Pole).

22 September 2010, Worcs (T. O'Neill, K. Green, T. Locke).

Technical meetings

30 November 2010, Worcs (K. Green, N. Dunn, J. Dew, J. Pecina).

Acknowledgements

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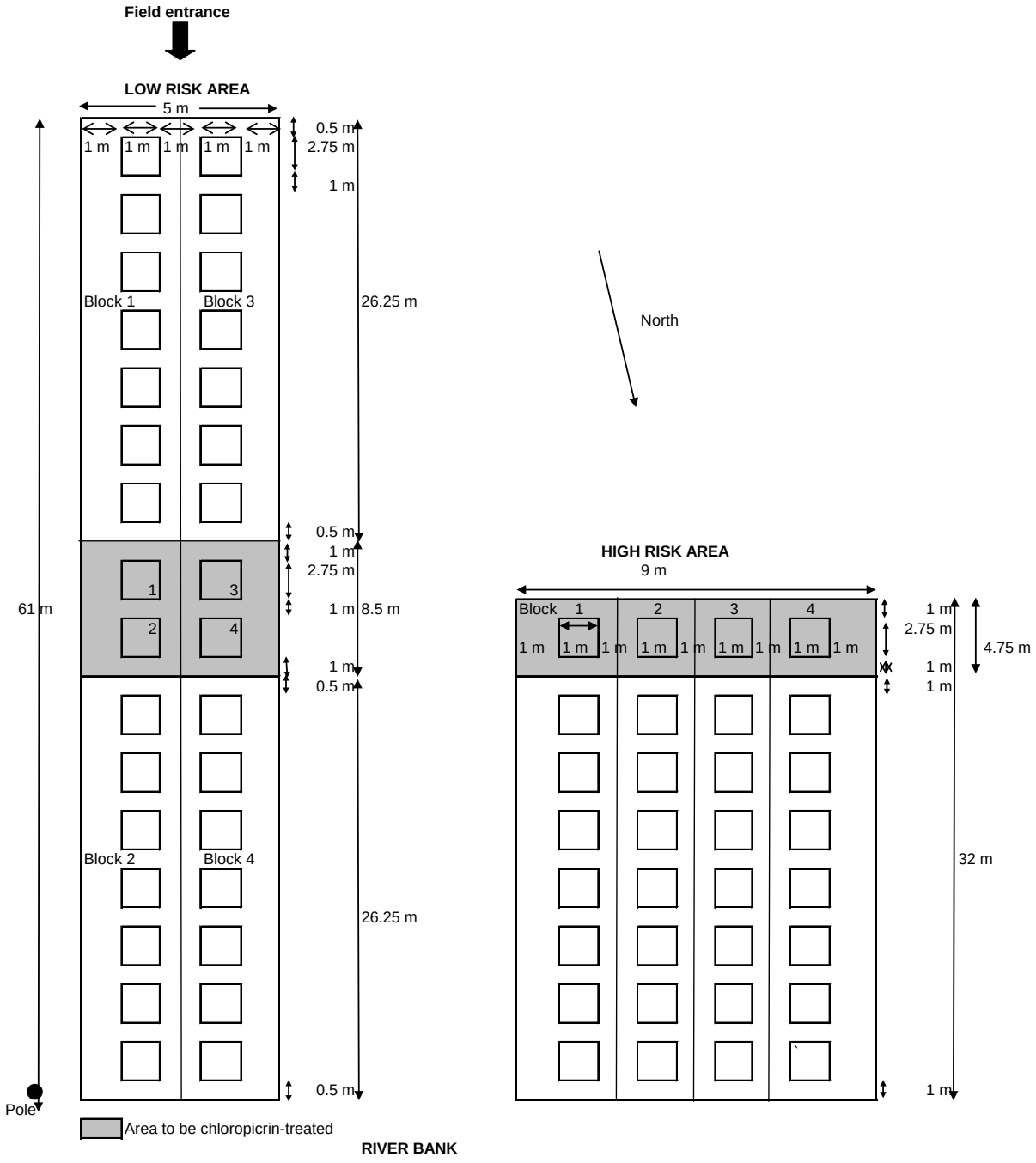
Appendix 1. Field trial plans

XBM 5539: SORBUS REPLANT TRIAL - FP MATTHEWS LTD

GRID REFERENCE: SO 568 677



Appendix 2. Monthly rainfall records for host farm, Worcestershire

Month (2010)	Total rainfall (mm)
April	19
May	40
June	51
July	33
August	69
September	51
October	82

Appendix 3. Trial diaries

Sorbus, Tenbury Wells, 2010

01 Oct 09	Trial marked out and Chloropicrin injected into soil
08 Oct 09	Basamid and Mustard meal applied to plots
20 Oct 09	Plastic removed from Chloropicrin plots
19 Nov 09	Plastic removed from Basamid/Mustard meal plots
16 Mar 10	SMN Soil sampling from Low and High risk areas
13 Apr 10	Herbicide application: Basta (glufosinate-ammonium) 5lLts/Ha applied via knapsack, sunny with light breeze
27 Apr 10	Trial Planted. Treatments 5, 6, 7 applied.
30 Apr 10	Herbicide application: Butisan S (metazachlor) 2.5Lts/Ha applied via knapsack, light rain
30 Apr 10	Herbicide application: Flexidor 125 (isoxaben) 2.0 Lts/Ha applied via knapsack, light rain
24 May 10	Fungicide application: Systhane 20EW (myclobutanil) 450mls/Ha
25 May 10	Treatment 6, 7, 8 applied.
03 Jun 10	Trial irrigated
22 Jun 10	Treatments 5, 6, 7, 8 applied
23 Jun 10	Fungicide application: Topas (penconazole) 500mls/Ha
23 Jun 10	Insecticide application: Calypso (thiacloprid) 250mls/Ha
25 Jun 10	Herbicide application: Basta (glufosinate-ammonium) 5lts/Ha applied via knapsack, sunny. spot treatment only.
02 Jul 10	Sorbus vigour score
19 Jul 10	Fungicide application: Systhane 20EW (myclobutanil) 450mls/Ha
19 Jul 10	Insecticide application: Aphox (primicarb) 560gms/Ha
02 Aug 10	Sorbus vigour score
12 Aug 10	Budding on Sorbus plots
13 Aug 10	Herbicide application: Basta (glufosinate-ammonium) 5lts/Ha applied via knapsack, sunny. spot treatment only.
18 Aug 10	Fungicide application: Topas (penconazole) 500mls/Ha
17 Sep 10	Fungicide application: Topas (penconazole) 500mls/Ha
12 Oct 10	Foliage weights cut from plots
24 Mar 11	5 trees dug up from T1 and T2 at high risk site and roots sent to ADAS Boxworth
31 Mar 11	Root sections plated on P5ARP and PDA+S at Boxworth

Malus

9/7/2010	Malus marking out and treatments applied
2/7/2010	Malus vigour score
2/8/2010	Malus vigour score
12/10/2010	Malus foliage weights

Appendix 4. Low risk area tree weights - 2010

XBM5539 Sorbus Replant Disease
Low Risk Area Plan

Plot 1,	T5,	FW 107	Rep 1	Plot 17,	T3,	FW 76	Rep 3
Plot 2,	T3,	FW 78		Plot 18,	T5,	FW 76	
Plot 3,	T8,	FW 67		Plot 19,	T6,	FW 55	
Plot 4,	T7,	FW 108		Plot 20,	T4,	FW 64	
Plot 5,	T4,	FW 136		Plot 21,	T8,	FW 83	
Plot 6,	T1,	FW 152		Plot 22,	T7,	FW 126	
Plot 7,	T6,	FW 166		Plot 23,	T1,	FW 128	
Plot 8,	T2,	FW 237		Plot 24,	T2,	FW 213	
Plot 9,	T2,	FW 221	Rep 2	Plot 25,	T2,	FW 219	Rep 4
Plot 10,	T5,	FW 184		Plot 26,	T4,	FW 176	
Plot 11,	T7,	FW 225		Plot 27,	T8,	FW 190	
Plot 12,	T4,	FW 167		Plot 28,	T6,	FW 216	
Plot 13,	T6,	FW 152		Plot 29,	T5,	FW 216	
Plot 14,	T1,	FW 196		Plot 30,	T7,	FW 216	
Plot 15,	T3,	FW 275		Plot 31,	T3,	FW 183	
Plot 16,	T8,	FW 182		Plot 32,	T1,	FW 187	

FW- Mean fresh weight taken from top growth (g)

T 1	Untreated	-
T 2	Custo-Fume	50 ml/m2
T 3	Basamid	57 g/m2
T 4	Mustard Meal – Biofence	250 g/m2
T 5	Novozymes MycorrhizaRoots	200 g/1L
T 6	RootMate granule/Plantmate WP	25 g +1.5 kg/ha
T 7	Agralan Revive	100 ml/10 L
T 8	Supplementary nitrogen	66.5 N/ha

Appendix 5. High risk area tree weights - 2010

XBM5539 Sorbus Replant Disease
High Risk Area Plan

Plot 1, T2, FW 218	Plot 9, T2, FW 205	Plot 17, T2, FW 267	Plot 25, T2, FW 172
Plot 2, T1, FW 107	Plot 10, T6, FW 101	Plot 18, T7, FW 121	Plot 26, T6, FW 113
Plot 3, T6, FW 74	Plot 11, T5, FW 71	Plot 19, T8, FW 68	Plot 27, T7, FW 115
Plot 4, T8, FW 34	Plot 12, T8, FW 52	Plot 20, T5, FW 37	Plot 28, T8, FW 38
Plot 5, T4, FW 41	Plot 13, T4, FW 31	Plot 21, T1, FW 29	Plot 29, T3, FW 50
Plot 6, T3, FW 49	Plot 14, T7, FW 53	Plot 22, T6, FW 43	Plot 30, T5, FW 40
Plot 7, T5, FW 35	Plot 15, T1, FW 37	Plot 23, T4, FW 39	Plot 31, T1, FW 27
Plot 8, T7, FW 34	Plot 16, T3, FW 52	Plot 24, T3, FW 59	Plot 32, T4, FW 46
Rep 1	Rep 2	Rep 3	Rep 4

FW- Mean fresh weight taken from top growth (g)

T 1	Untreated	-
T 2	Custo-fume	50 ml/m2
T 3	Basamid	57 g/m2
T 4	Mustard Meal – Biofence	250 g/m2
T 5	Novozymes MycorrhizaRoots	200 g/1L
T 6	RootMate granule/Plantmate WP	25 g +1.5 kg/ha
T 7	Agralan Revive	100 ml/10 L
T 8	Supplementary nitrogen	66.5 N/ha

Appendix 6. Trial diary – Sorbus seedling bioassay, 2010

Date	Comment
20/04/2010	Sorbus seed ordered from Forestart Ltd, provenance 403, which is the originating forest where the seed stock came from for the 2009 trial. Seed used in Tenbury Wells trial was not available
22/04/2010	20 x seeds per seed tray of Sorbus aucuparia sown into 20 seed trays filled with F2 + S compost.
	Placed onto capillary matting in GH4 at 15-20 degrees C
28/04/2010	Germination count carried out, 112 seedlings emerged
06/05/2010	Germination count carried out, 224 seedlings emerged to give 40 % germination rate as indicated by Forestart Ltd.
07/05/2010	Soil delivered from Rosemaund
18/05/2010	Seedlings moved to polytunnel 1 to 'harden' off before planting
21/05/2010	Soils tumbled and split into 2 lots and 1 half taken for Chloropicrin treatment by Custodian
	Fumigation in Eye, Suffolk
14/06/2010	Soils collected from Custodian Fumigation and placed into potting shed
15/06/2010	Cress tests set up
22/06/2010	Seedlings transplanted to soils, placed into polytunnel 2, as weather very hot, fleece wasn't used to cover the seedlings but the polytunnel sides were rolled down. Watered in around the base of each plant by hose
26/06/2010	Automatic misting started at 1:30 minutes 3 times a day
30/06/2010	1st seedling height assessment completed
05/07/2010	Watering increased to 2:30 mins, 3 times a day
13/07/2010	Trial fed with Vitafeed 214 at 1:100 dilution once a week from here on.
22/07/2010	Watering increased to 5 mins, 3 times a day
06/08/2010	6 week vigour assessment completed
24/08/2010	Height Assessment completed
14/09/2010	Height and Destructive Assessment completed - Roots to be plated
21/09/2010	Sorbus roots sterilised in disinfectant and plated out onto PDA + S - 4 root pieces per plate, and 5 plates per treatment.
27/09/2010	6 Day Assessment of Sorbus root plates completed. Plates moved to UV incubator to try and induce sporulation

Appendix 7. DNA multiscan results on Custo-Fume (A) and untreated (B) soil

Organisme	Sample A	Sample B
Plantpathogene schimmels		
Alternaria sp.	0	0
Athelia (Sclerotium) rolfsi	0	0
Botrytis sp.	0	0
Botrytis cinerea	0	0
Botrytis porri	0	0
Botrytis tulipae	0	0
Colletotrichum sp.	0	0
Colletotrichum acutatum	0	0
Colletotrichum coccodes	0	0
Coniothyrium fuckelii	0	0
Cylindrocladium sp.	0	0
Cylindrocarpon destructans	1	1
Didymella sp.	0	0
Fusarium sp.	3	2
Fusarium culmorum	0	0
Fusarium oxysporum	1	1
Fusarium sacchari	0	0
Fusarium solani	0	0
Geotrichum candidum	0	0
Myrothecium roridum	0	0
Phoma destructiva	0	0
Phomopsis scleriotioides	0	0
Plectosphaerella cucumerina	0	0
Pyrenochaeta lycopersici	0	0
Rhizoctonia solani	0	0
Sclerotinia sp.	0	0
Sclerotinia minor	0	0
Sclerotinia sclerotiorum	0	0
Sclerotinia trifoliorum	0	0
Thielaviopsis basicola	0	0
Verticillium sp.	0	0
Verticillium albo-atrum	0	0
Verticillium dahliae	0	0
Pythium sp.	0	3
Pythium aphanidermatum	0	0
Pythium dissotocum	0	0
Pythium irregulare	0	0
Pythium polymastum	0	0
Pythium sylvaticum	0	0

Pythium ultimum	0	0
Phytophthora sp.	0	0
Phytophthora cactorum	0	0
Phytophthora capsici	0	0
Phytophthora cinamomi	0	0
Phytophthora citricola	0	0
Phytophthora cryptogea	0	0
Phytophthora drechsleri	0	0
Phytophthora infestans	0	0
Phytophthora nicotianae	0	0
Phytophthora ramorum	0	0
Nuttige organismen		
Trichoderma sp	3	1
Trichoderma asperellum	0	0
Trichoderma hamatum	0	0
Trichoderma harzianum	0	0

Index:

0 = not detectable

1 = light infestation

2 = moderate infestation

3 = high infestation