

# **Grower Summary**

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## **HNS 168**

Rosaceous trees: evaluation of  
treatments for control of replant  
disease in *Sorbus aucuparia*

Final 2011

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HDC is a division of the Agriculture and Horticulture Development Board.

**Project Number:** HNS 168

**Project Title:** Rosaceous trees: evaluation of treatments for control of replant disease in Sorbus aucuparia

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**Contractor:** ADAS

**Industry Representative:** Dr Tom Locke

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## 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 disinfecting 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 <i>Sorbus</i> replant disease based on cropping history	Soil treatment	% R after	
		7 weeks	11 weeks
'Low risk'	Autoclave	143	<b>171</b>
	Custo-Fume	122	142
'High risk'	Autoclave	<b>198</b>	<b>238</b>
	Custo-Fume	<b>169</b>	<b>153</b>

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	
	Current <i>Sorbus</i>	Recent <i>Sorbus</i>	Recent treatment	CP		(% R and interpretation)	
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<sup>2</sup>; and 'Biofence' Caliente mustard meal (*Brassica carinata*) at 250 g/m<sup>2</sup>. Soil temperature was around 11°C 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<sub>3</sub>)<sub>2</sub>) 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 status as pesticide (August 2011)	approval as a treatment	Active ingredient	Mean fresh weight per plant of top growth (g) <sup>a</sup>	
				High risk site	Low risk site
1. Untreated	-	-	-	51	166
2. Custo-Fume <sup>b</sup>	Label approval		Chloropicrin <sup>f</sup>	218	223
3. Basamid	Label approval		Dazomet	54	153
4. Biofence pellets	Not applicable <sup>c</sup>		mustard meal	40	136
5. Novozyme Roots	Exempt		Mycorrhiza	47	146
6. PlantMate	Experimental <sup>d</sup>		<i>Trichoderma harzianum</i>	84	148
7. Agralan Revive	Experimental <sup>e</sup>		<i>Bacillus subtilis</i>	82	169
8. Extra N	Not applicable <sup>c</sup>		350 kg Ca(NO <sub>3</sub> ) <sub>2</sub>	49	131

<sup>a</sup> Includes weight of leaves. <sup>b</sup> Replicate plots were not randomised. <sup>c</sup> Fertiliser products, out of scope of pesticides legislation. <sup>d</sup> Used under an Experimental Permit. <sup>e</sup> Out of scope of

pesticides legislation at the time it was used in this project. †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.