Field grown trees: evaluation of chemical and biological pre-plant soil treatments for control of verticillium wilt

HNS 137

March 2008

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The results and conclusions in this report are based on an experiment conducted over one year. The conditions under which the experiment was carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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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

Verticillium wilt in *Acer* and *Tilia* was significantly reduced by pre-plant soil treatment with Chlorofume or Basamid/Sistan but not by sudan grass or Biological Soil Disinfestation (BSD); low soil levels of *V. dahliae* resulted in infection of *Acer* but not of *Tilia*, whereas high levels resulted in infection of both species.

Background and expected deliverables

The value of the 1,535 ha of ornamental tree stock in England and Wales is around £29.7 million (Defra Basic Horticultural Statistics, 2003). Several of the subjects grown are susceptible to the serious, soil-borne fungal disease verticillium wilt, notable examples being some species of *Acer*, *Tilia, Fraxinus* and *Catalpa*. The causal fungus, *Verticillium dahliae*, is widespread in UK soils. Until 2004, around 15 ha of land were treated each year with methyl bromide prior to planting trees, primarily to reduce the risk of verticillium wilt.

In the UK and other developed countries, the pre-plant application of methyl bromide to soil was recently prohibited. From 1 January 2007, pre-plant soil disinfestation for tree production must use a means other than methyl bromide. Without an effective alternative, the losses incurred to verticillium wilt are likely to increase substantially, effectively preventing the production of certain tree species in the UK on a commercial scale. Container production is not a viable option for growing trees to a large size.

The overall aim of this project is to identify one or more alternatives to methyl bromide for soil disinfestation in the field-grown tree industry. The treatment should be applicable broadacre and provide effective control of *V. dahliae* to sufficient depth to enable economic production of a crop growing for at least 4 years.

Specific objectives are:

- To determine the effectiveness of two chemical and two biological soil treatments in reducing inoculum of *V. dahliae* in soil and the control of verticillium wilt in two tree species.

- To determine the effect of the soil treatments on nematode populations and initial weed control.

- To determine the effect of the soil treatments on tree growth.

- To determine if there is any basis for developing strategic planting of two different tree species based on levels of *V. dahliae* in the soil.

- To determine if there is any obvious relationship in soil infestation levels of *V. dahliae* as determined by conventional plate testing compared with a molecular method (PCR).

Summary of the project and main conclusions

Details of soil disinfestation treatments

Full details are given in the March 2006 report. Briefly, a large-plot field experiment was established in 2005 on land infested with *V. dahliae.* Soil treatments were:

1. Untreated control (fallow, with weeds controlled by soil cultivation).

2. Sudan grass (cv. Nigrum) grown on site for 3 months, incorporated on 6 September as a green manure. No soil cover.

3. Biological Soil Disinfestation (BSD). Italian rye-grass cv. Danergo was grown on plots and incorporated on 9 August (at approximately 5.7 kg fresh weight/m^2), to 35 cm depth by spading machine, irrigated to field capacity, and covered with oxygen-impermeable plastic to create anaerobic conditions in the soil.

4. K&S Chlorofume (99.5% chloropicrin) injected to 35 cm depth at 40 mL/m^2 (maximum permitted rate) by contractor on 26 August.

5. Basamid (98% dazomet) applied at 45 g/m^2 and incorporated to 20 cm depth, and Sistan 51 (51% metam sodium) injected at 25–35 cm depth at 90 mL/m^2 (= 46 mL/m^2 of metam sodium, maximum permitted rate) by contractor on 26 August.

Before treatments were applied, soil samples from 25 plots were tested for *V. dahliae* and nematodes. Levels of *V. dahliae* ranged from 4.9 to 38.2 colony forming units (cfu) per g of soil (mean 15.1) while levels of nematodes were low (<300/litre).

Efficacy of soil treatments against V. dahliae

Reduction of *V. dahliae* levels in soil:

In January 2008, the mean level of *V. dahliae* in untreated soil (43.6 cfu/g) had increased over that found a year earlier (25.9 cfu/g) (Table 1). The mean levels of *V. dahliae* in the BSD, Chlorofume or Basamid/Sistan 51 treated soils were significantly lower than in untreated soil. The two chemical treatments were particularly effective. Sudan grass no longer had any significant effect on the level of *V. dahliae* in soil.

The reason for the increase in levels of *V. dahliae* between January 2007 and January 2008 is unknown. A possible explanation is release of the fungus from decaying roots of infected *Acer* and *Tilia* trees.

Table 1: Effect of soil treatment on the level of *V. dahliae* in soil (cfu/g)

Treatment	Before treatment	After treatment		
	March 05	January 06	January 07	January 08
Untreated	16.0	33.8	25.9	43.6
Sudan grass	15.5	23.0	11.1	43.1
BSD	16.1	10.1	5.7	27.7
Chlorofume	14.3	2.5	0.6	5.6
Basamid/Sistan 51	13.6	0.4	1.1	2.2

Control of infection of trees by *V. dahliae:*

In April 2006, the plots were planted with *Acer platanoides* ‘Emerald Queen’, *Tilia cordata* ‘Greenspire’ and *Sorbus aria* ‘Majestica’ on *Sorbus intermedia* rootstock. The soil between and around trees was treated with herbicide and cultivated to control weeds. In October 2006, one third of the trees in each plot were cut down and the stem bases were tested for infection by *V. dahliae.* Although none of the trees showed definitive symptoms of verticillium wilt at this time, *V. dahliae* was recovered from 33% of *Acer* trees grown in untreated soil and from fewer trees grown following soil treatment with Basamid/Sistan (3%) or Chlorofume (17%). Neither sudan grass nor BSD reduced the incidence of infection.

In early autumn 2007, after two growing seasons, symptoms of verticillium wilt appeared in some of the *Acer* and *Tilia* trees. The incidence of leaf yellowing and bark splitting in *Acer*, suggestive of verticillium wilt, was reduced by the two chemical treatments (Table 2). There was no significant treatment effect on leaf yellowing in *Sorbus* or *Tilia*.

Table 2: Effect of pre-plant soil treatment on symptoms of verticillium wilt in *Acer* and leaf yellowing in *Sorbus* and *Tilia* – autumn 2007

	Treatment	Mean % plants with presumptive verticillium wilt symptoms			
		Acer		Sorbus	Tilia
		Leaf yellowing (Sep)	Bark split (Oct)	Leaf yellowing (Sep)	Leaf yellowing (Sep)
1.	Untreated	33.9	22.1	0.7	2.7
2.	Sudan grass	31.2	23.3	0	4.7
3.	BSD	24.6	19.1	0	0.7
4.	Chlorofume	12.1	7.9	1.6	0.6
5.	Basamid/Sistan 51	6.7	6.1	1.4	0

On 8 October 2007, one third of the original number of trees in each plot was cut down and stem bases were tested for infection by *V. dahliae*. The fungus was recovered from 34.7% of *Acer* trees grown in untreated soil and from significantly fewer trees following soil treatment with Basamid/Sistan (16.5%) or Chlorofume (15.6%). The same two treatments reduced the incidence of infection in *Tilia* from 18% to 0% (Table 3). No *V. dahliae* was recovered from the *Sorbus* stem bases tested.

Table 3: Effect of pre-plant soil treatments on recovery of *V. dahliae* from tree stem bases – autumn 2007

Treatment		Mean % trees infected	
		Acer	Tilia
1.	Untreated	34.7	18.1
2.	Sudan grass	43.7	19.7
3.	BSD	47.2	8.9
4.	Chlorofume	15.6	0.0
5.	Basamid/Sistan 51	16.5	0.0

Soil level of V. dahliae and risk of verticillium wilt

There was evidence that the incidence of *Acer* trees showing presumptive symptoms of verticillium wilt in autumn 2007, 18 months after planting, was related to the level of *V. dahliae* in the soil at planting, with an increase of symptoms at soil levels above 2.7 cfu/g (Table 4). Leaf yellowing in *Tilia* (presumptive verticillium wilt) occurred at a much lower incidence than in *Acer*, and mostly in plots with a *V. dahliae* soil level of > 10 cfu/g at planting.

The incidence of *Acer* trees definitely infected by *V. dahliae*, as measured by recovery of the fungus from stem bases, also appeared to be related to the level of soil infestation. The incidence of infected trees was greater at levels above 2.9 cfu/g (36–38%) than below 0.7 cfu/g (10–13%).

Table 4: Effect of soil level of *V. dahliae* at planting on occurrence of verticillium wilt symptoms in Acer and Tilia - autumn 2007

V. dahliae in soil (cfu/g)*	Acer leaf yellowing	Acer bark splitting	Tilia leaf yellowing
0	3.3	6.7	0
0.1–0.7	9.5	6.9	0
2.9–10.0	26.4	17.9	0.6
>10.0	32.3	23.9	4.1

*There were no soils with *V. dahliae* levels between 0.7 and 2.9 cfu/g.

Effect of soil treatments on tree growth

The extension growth in 2007 of *Acer* and *Tilia* was significantly increased by all four soil treatments, Chlorofume having the greatest effect on *Acer* and BSD on *Tilia.* Chlorofume or Basamid/Sistan significantly increased stem circumference of *Sorbus.* The growth response in *Sorbus* to these treatments may have been due to control of *Sorbus* replant disease or to other factors (e.g. nutrient release), as no *V. dahliae* was isolated from this species. There was a trend for reduced growth with increasing soil levels of *V. dahliae* in all three species but the correlation was poor.

Effect of soil treatments on nematodes

Soil sampled in May 2007, 18 months after treatment, contained low levels of stubby root and stunt/spiral nematodes. Levels were significantly reduced by Chlorofume or Basamid/Sistan and not by the two biological treatments.

Quantifying V. dahliae in soil by a molecular test (PCR)

Soil samples from the field experiment in Hampshire were tested for *V. dahliae* DNA using a TaqMan PCR test. Results were inconsistent. From examination of the data it was concluded that extraction of nucleic acids from soils was inefficient and that most of the signal obtained was due to an organism other than *V. dahliae*. No further work will be done on molecular testing of soil for *V. dahliae* in this project.

Financial benefits

In April 2005, discussion with nine UK tree growers with a total annual production of over 92,000 *Acer* trees, indicated that losses to verticillium wilt in field-grown trees ranged from 5% to 50% (mean: 28.3%). Assuming an average sale value of £14 per tree, the annual sales loss for these nine growers is £364,933 and the annual production cost loss is £182,467. Identification of an effective method for soil disinfestation is therefore likely to have substantial financial benefits for UK growers of verticillium-susceptible trees.

In autumn 2007, a field in Hampshire with no recorded history of cropping trees, potatoes or other verticillium-susceptible species, that had been down to grass for many years, was severely affected by verticillium wilt in *Acer* and *Tilia* planted on the site in 2005. Several hundred trees were badly affected. Soil taken from mid-way between rows of trees was tested for *V. dahliae* by ADAS and found to contain high levels (41.6 cfu/g). This damaging outbreak of verticillium wilt highlights the damage that the disease can cause. Species affected were *Acer* 'Emerald Queen', *Acer campestre, Tilia cordata* and *Tilia euchlora*.

Action points for growers

- Do not plant *Acer platanoides* on land where *V. dahliae* has been detected. After just 6 months, around 33% of trees planted on untreated land infested with 7–62 cfu/g had become infected by *V. dahliae*; even on land with 0.1–0.7 cfu/g, 10% of trees had become infected.

- *Tilia cordata* can tolerate a higher soil infestation of *V. dahliae* than *Acer plantanoides*. After 18 months, 18% of *Tilia* trees planted on land infested with 7–62 cfu/g had become infected; compared with 33% of *Acer* trees. On land with 0.1–0.7 cfu/g, no *Tilia* trees had become infected by this time, but the project has two more years to run.

- Soil treatment with Basamid/Sistan 51 or Chlorofume, at the rates described in this report, can reduce soil levels of *V. dahliae* by over 90%, a result only a little inferior to that expected by methyl bromide. However, results to date suggest this degree of reduction is insufficient to prevent substantial infection of *Acer* if *V. dahliae* is still detected in soil after treatment (i.e. at 0.1 cfu/g or more, the minimum level of detection in this work).

- BSD applied as described in this report reduced soil levels of *V. dahliae* by around 70%.

- Chlorofume or Basamid/Sistan, at the rates used in this experiment, prevented infection of *Tilia cordata* by *V. dahliae* in the first 18 months after planting; the levels of *V. dahliae* remaining after these soil treatments ranged from 0 to 0.7 cfu/g.

- BSD applied as described in this report significantly reduced, but did not prevent, infection of *Tilia cordata.* Results to date suggest *T. cordata* should not be planted on land with *V. dahliae* levels greater than 0.7 cfu/g.

- Many dicotyledonous weeds that are common in the UK (e.g. fat hen, shepherd's purse), and several crops commonly grown in the UK, can act as hosts of *V. dahliae*. **It is strongly recommended** that a pre-plant soil test, to determine the soil levels of *V. dahliae,* is conducted prior to planting a susceptible crop, such as *Acer,* even if there is no recent past history of a susceptible crop having been grown on the land.

- If land has been cropped with *Tagetes* (e.g. for control of nematodes), do not use the field for production of *Acer* or other species highly susceptible to *V. dahliae* unless the field has been adequately tested and found to be free of the fungus.

- From the results obtained to date, there is no evidence that *Sorbus aria* 'Majestica' on *Sorbus intermedia* rootstock is susceptible to verticillium wilt.

- Chlorofume or Basamid/Sistan soil treatments significantly increased the circumference of *Sorbus* trees and appeared to increase extension growth, even though there was no evidence that the trees were infected by verticillium wilt. It is possible that this growth response was due to control of *Sorbus* replant disease.

- Chlorofume or Basamid/Sistan can significantly reduce soil levels of stubby root and stunt/spiral nematodes. The biological treatments used in this work were ineffective against nematodes.

SCIENCE SECTION

Introduction

In the first stage of this experiment, two chemical soil disinfestation treatments (Basamid/Sistan 51 or Chlorofume) or one biological treatment (Biological Soil Disinfestation) significantly reduced the levels of *V. dahliae* in soil; a second biological treatment (sudan grass) was ineffective. The levels of *V. dahliae* in January 2006, three months after soil treatment, ranged from nil to 62.2 cfu/g soil.

In the second stage of the experiment, the objective is to determine how two verticillium-susceptible tree species (*Acer* and *Tilia*) respond to different levels of *V. dahliae* in the soil. No field symptoms were observed in autumn 2006, after one growing season through a dry summer. In 2007, trees were examined for field symptoms of verticillium wilt and for infection in the stem base. Additionally, the effect of the soil disinfestation treatments on growth of *Sorbus aria*, a species usually considered field-resistant to verticillium wilt, but susceptible to replant disease, was examined.

Materials and methods

<u>Occurrence of verticillium wilt symptoms – autumn 2007</u>

Field assessments:
All trees were assessed on 11 September to determine the occurrence of leaf yellowing and necrosis, and on 8 October to determine bark splitting on the main stem, suggestive of verticillium wilt. Leaf yellowing severity was assessed on a 0–4 scale; trees with a score of 3 or 4 were considered to be showing symptoms of verticillium wilt. The categories were:

0 – Nil
1 – Slight (<10% leaves discoloured or brown)
2 – Moderate (10–50% leaves discoloured, generally mottled)
3 – High (51–90% leaves brown)
4 – Severe (91–100% leaves brown)

Bark splitting in *Acer* stems was assessed on a 0 – 2 scale; trees with a score of 2 were considered to be showing symptoms of verticillium wilt. The categories were:

0 – Nil
1 – Bubbling and small splits
2 – Definite cracking of bark

Tree growth was determined on 13–14 November by measuring the extension growth of the main leader on 15 trees of each species per plot (the central plant in each group of three originally planted). The trunk circumference at 50 cm above soil level was measured on the same trees.

The eastern-most tree in each group of three was cut off at ground level and a 30 cm length of stem was removed from the stem base at around 20 cm height (above the graft) for laboratory testing for *V. dahliae.*

Laboratory tests:

Stem bases were tested for *V. dahliae* as described previously (see Year 2 report). All of the sampled *Acer* and *Tilia* stem bases were tested, together with all *Sorbus* stems from plot 25 (a plot with high soil levels of *V. dahliae*). Stem bases were also assessed for staining of wood, as previously.

<u>Levels of *V. dahliae* in the soil – January 2008</u>

Soil samples were taken from each plot on 14 January 2008 and tested for *V. dahliae*. Samples were collected and tested as previously described.

<u>Levels of nematodes in the soil</u>

Soil samples were taken from each plot on 30 May 2007 and tested for nematodes at ADAS High Mowthorpe.

<u>Determination of *V. dahliae* in soil by a PCR test</u>

As part of HDC project SF 70, a real-time polymerase chain reaction (PCR) test to determine levels of *V. dahliae* DNA in soil was developed. Soil samples collected in this project, with known levels of *V. dahliae* as determined by conventional plating onto agar, were re-tested using the PCR test.

Fifty samples of soils were received at EMR from the field site in Hampshire. These represented each of the 25 field plots before and after soil disinfestation treatment. The soils had been previously fractionated as for the wet-sieve soil analysis protocol. From each sample, DNA was extracted using the MoBio Power Soil kit and protocol described in the HDC SF 70 final report (2007). Subsequent to extraction, the material was passed through a polyvinylpyrrolidone column in order to remove PCR inhibitors. The final extract was amplified using *V. dahliae* specific primers (Attalah *et al.,* 2007).

Statistical analysis

Results were examined by ANOVA or by regression analysis using the logit link function where data were binomially distributed.

Results and discussion

Field symptoms of verticillium wilt – autumn 2007

Almost all trees appeared very healthy when trees were inspected on 30 May 2007. Just three were growing poorly and these had bark splits suggestive of verticillium wilt. By 25 September 2007, symptoms of verticillium wilt in *Acer* were found quite readily.

When assessed in October, longitudinal bark splitting on the main stem suggestive of *V. dahliae* infection was found in around 22% of *Acer* trees on untreated soil and not at all in *Tilia* or *Sorbus*. The occurrence of bark splits (index 2) was significantly reduced by the two chemical soil treatments (Table 5). Leaf yellowing occurred in around one third of *Acer* and occasional *Tilia* trees on untreated soil. Leaf yellowing (index 3 & 4) in both species appeared to be reduced by the two chemical soil treatments but was not statistically significant at p=0.05. The occurrence of both bark splits and leaf yellowing in *Acer* was significantly related to the level of *V. dahliae* in the soil at planting (Table 6).

Table 5: Effect of pre-plant soil treatment on occurrence of bark splits and leaf yellowing and/or necrosis of *Acer* according to pre-plant soil treatment – field assessment, October 2007

Treatment	Mean % trees		
	Acer		Tilia
	Split bark	Leaf yellowing	Leaf yellowing
1. Untreated	22.1	33.9	2.7
2. Sudan grass	23.3	31.2	4.7
3. BSD	19.1	24.6	0.7
4. Chlorofume	7.9	12.1	0.6
5. Basamid/Sistan	6.1	6.7	0
Significance	0.004	0.076	0.215

(Split bark: index 2; Leaf yellowing: index 3 or 4)

Table 6: Occurrence of bark splits and leaf yellowing with necrosis in *Acer* according to soil levels of *V. dahliae* prior to planting – field assessment, October 2007

Level of *V. dahliae* (cfu/g) in January 2006*	Number of plots	Mean % *Acer* trees with	
		Leaf yellowing	Bark split
0	1	3.3	6.7
0.1–0.7	8	9.5	6.9
2.9–10.0	6	26.4	17.9
>10.0	9	32.3	23.9
Significance		<0.001	<0.001
Regression coefficient		42.3	47.2

*There were no soils with *V. dahliae* levels between 0.7 and 2.9 cfu/g.

<u>Isolation of *V. dahliae* from trees</u>

Overall, *V. dahliae* was isolated from the stem bases of 31.5% of *Acer* trees, 9.4% of *Tilia* trees and from no *Sorbus* trees. Given the low level of *V. dahliae* recorded in the trees pre-planting (2% in Acer; see year 2 report), and the large differences in the incidence of infection associated with different soil treatments, it seems probable that the majority of infection in trees arose from *V. dahliae* in the soil.

The recovery of *V. dahliae* from *Acer* trees destructively sampled in October 2007 was broadly similar to the levels recovered in 2006 (Table 7). The greatest change was in trees grown on land treated with Basamid/Sistan, where recovery increased from 2.6% to 16.5%. In 2007, there were significant differences between treatments (p=0.010) but not between blocks (p=0.134). Chlorofume or Basamid/Sistan soil treatment in 2005 resulted in significantly fewer trees becoming infected with *V. dahliae* by 2007 than in untreated soil. As in 2006, the sudan grass and BSD soil treatments gave no reduction in the incidence of infected trees.

The combined data in Table 7 represent isolation results for around 30 trees per plot. There were highly significant (p<0.001) differences between treatments but no significant differences (p=0.154) between blocks. The two chemical treatments resulted in significant control of infection by *V. dahliae*.

Infection of *Tilia* by *V. dahliae* was significantly reduced (p=0.002) by pre-plant soil treatment with Chlorofume or Basamid/Sistan (Table 8). Although not eliminating *V. dahliae* from the soil, these two treatments reduced infestation to a level (0.1–0.7 cfu/g) such that no trees had become infected 18 months after planting. Sudan grass was ineffective, while the BSD treatment appeared to reduce the incidence of affected trees by around 50%.

Table 7: Effect of pre-plant soil treatment on recovery of *V. dahliae* from *Acer* stem bases

Treatment	Mean % trees *V. dahliae* isolated:		
	2006	2007	Combined
1. Untreated	32.8 (10.3)	34.7 (6.7)	33.7 (5.0)
2. Sudan Grass	34.1 (10.4)	43.7 (7.0)	38.9 (5.1)
3. BSD	42.1 (10.8)	47.2 (7.0)	44.7 (5.2)
4. Chlorofume	18.5 (10.2)	15.6 (5.6)	16.7 (4.5)
5. Basamid/Sistan	2.6 (3.5)	16.5 (5.3)	9.4 (3.1)
Significance (15 df)	0.043	0.010	<0.001

( ) standard error

Table 8: Effect of pre-plant soil treatment on recovery of *V. dahliae* from Tilia stem bases

Treatment	Mean % trees *V. dahliae* isolated
1. Untreated	18.1 (5.1)
2. Sudan grass	19.7 (5.1)
3. BSD	8.9 (3.7)
4. Chlorofume	0.0 (0)
5. Basamid/Sistan	0.0 (0)
Significance (15 df)	0.002

( ) standard error

<u>Wood staining</u>

Pale green to brown staining in *Acer* is generally considered to be a symptom of infection by *V. dahliae.* Although the incidence of staining in trees from untreated, sudan grass and BSD treatments appeared to be greater than from trees grown following Chlorofume or Basamid/Sistan soil treatment, the differences were not significant (p=0.20) (Table 9).

The association of *V. dahliae* recovery from stained and unstained wood was examined. Overall, *V. dahliae* was recovered from 21.9% of stem bases with stained wood and from 9.6% of stem bases with unstained wood. There were no significant differences between soil treatments (Table 10).

Staining in *Tilia* stems ranged in severity from 0.8 to 1.2 on a 0–3 index. Soil treatment had no significant effect on the severity of staining (p=0.142).

Table 9: Occurrence of wood staining in *Acer* stem bases – 2007

Treatment		Mean % stems with definite wood staining (standard error)
1.	Untreated	45.3 (11.7)
2.	Sudan grass	37.9 (11.4)
3.	BSD	44.1 (11.5)
4.	Chlorofume	15.1 (9.4)
5.	Basamid/Sistan	17.6 (8.9)
Significance (15 df)		NS

( ) standard error

Table 10: Association of wood staining and isolation of *V. dahliae* from *Acer* - 2007

Treatment		Mean % stems where *V. dahliae* was isolate:	
		Wood stained	Wood not stained
1.	Untreated	23.3 (8.4)	11.3 (4.8)
2.	Sudan grass	31.1 (9.2)	12.6 (4.9)
3.	BSD	33.2 (9.4)	14.1 (5.2)
4.	Chlorofume	8.3 (6.1)	7.2 (3.9)
5.	Basamid/Sistan	13.5 (6.8)	2.8 (2.5)
Mean		21.9	9.6
Significance (15 df)		0.214	0.343

( ) standard error

<u>Association of field symptoms with isolation of *V. dahliae*</u>

Recovery of *V. dahliae* from the stem bases of *Acer* trees was not tightly related to the occurrence of field symptoms of verticillium wilt (leaf yellow index >2 or bark split index 2) (Table 11). Although *V. dahliae* was recovered more commonly from trees with field symptoms (42%) than from those without symptoms (29%) as expected, the fungus was not recovered from some trees with field symptoms. Possibly some of the leaf yellowing scored as presumptive verticillium wilt was due to some other cause, or to infection in the tree below the level at which the stem base section was taken for the isolation test.

Table 11: Association of presumptive verticillium wilt symptoms in *Acer* with recovery of *V. dahliae* from the stem base of these trees - 2007

	Number of trees in each category	
	Leaf yellowing and/ or bark split	No symptoms
V. dahliae isolated	34/81 (42%)	77/262 (29%)

(5 samples missing from stem base isolation; 12 from leaf yellowing assessment)

<u>Effect of soil infestation level with *V. dahliae* on occurrence of infection in Acer and Tilia trees</u>

The effect of the level of *V. dahliae* in the soil at planting on the incidence of trees that became infected with the fungus was examined. When the data for *Acer* stem base isolation in 2006 and 2007 are combined, it is seen that the level of tree infection is around three times greater at soil infestation levels of more than 2.9 cfu/g, compared with soil levels of less than 0.7 cfu/g (Table 12). A regression of soil infestation level (log *V. dahliae*) in January 2006, just before planting, against the total proportion of *Acer* trees infected was significant ($p<0.001$) and accounted for 38% of the variance.

For *Tilia*, no *V. dahliae* was isolated from trees grown in soil with 0.7 cfu/g or less. These results indicate that the sensitivity of *T. cordata* to *V. dahliae* was less than that of *A. plantanoides*. While it is unsafe to plant *A. platanoides* on land where any *V. dahliae* is detected in the soil, the results obtained to date in this project indicate that it is safe to plant *T. cordata* where the soil level of *V. dahliae* is 0.7 cfu/g, or less, as measured by the ADAS standard agar plate test. Note however that the project still has 2 years to run and this conclusion may change.

Table 12: Effect of soil infestation level with *V. dahliae* on occurrence of recovery of *V. dahliae* from *Acer* trees – October 2007

*V. d*ahliae level (cfu/g) in soil – Jan 06*	No. plots in category	No. plots with infected trees in 2007	Mean % trees infected/plot	
			2006	2006 + 2007
0	1	1	20.0	10.0
0.1 – 0.7	8	5	16.1	12.9
2.9 – 10.0	6	9	47.1	37.8
>10.0	9	8	37.8	36.3

*There were no soils with *V. dahliae* levels between 0.9 and 2.7 cfu/g.

Effect of soil treatment on tree growth

The extension growth in 2007 of *Acer* and *Tilia* was significantly increased by all four pre-plant soil disinfestation treatments (Table 13). Chlorofume resulted in the greatest increase in *Acer* while BSD resulted in the greatest increase in *Tilia*. There was no significant difference between the four soil treatments in either species. The two chemical soil treatments appeared to increase extension growth of *Sorbus*, but the difference was not quite significant at the 5% level.

The two chemical soil treatments significantly increased the circumference of the *Sorbus* trees (Table 14). There was a marked trend of increased stem circumference with soil treatment in *Acer* and *Tilia*, although the differences were not quite significant at the 5% level (Table 14).

<u>Effect of soil infestation with *V. dahliae* on tree growth</u>

The growth of trees was examined with respect to the level of *V. dahliae* in the soil at planting in 2006 and as determined in January 2007. Although there was a trend for reduced growth with increasing levels of *V. dahliae* in the soil, there was not a good fit for any of the parameters (Table 15). The strength of the correlations was not in the order *Acer* > *Tilia* > *Sorbus*. This may be because: a) *V. dahliae* does not significantly affect growth of surviving trees; b) there are other pathogens in the soil affecting tree growth; c) pre-plant soil disinfestation treatments influence growth in ways additional to killing soil-borne pathogens (e.g. by nutrient release and by altering the soil microorganism populations) or possibly for other reasons.

For each tree species, extension growth was positively correlated with stem circumference, the correlation being greater for *Tilia* (0.84) and *Sorbus* (0.74) than for *Acer* (0.55). Possibly the low value for *Acer* is a reflection of disturbed growth due to infection by *V. dahliae.*

No symptoms of verticillium wilt were observed in *Sorbus* in the experiment, and no *V. dahliae* was recovered from the stem bases of sampled trees, consistent with reports that this species is not susceptible. However, as reported above, the two chemical soil disinfestation treatments significantly increased tree circumference, and appeared to increase extension growth, while the two biological soil treatments (sudan grass and BSD) did not have this effect. The cause of this response to chemical soil disinfestation is unknown; possibly on untreated soil the *Sorbus* trees are affected by *Sorbus* replant disease.

Table 13: Effect of pre-plant soil treatment on extension growth in 2007

Treatment	Extension growth (cm)		
	Acer	Sorbus	Tilia
Untreated	29.0	32.2	62.1
Sudan grass	68.2	32.9	77.2
BSD	56.9	39.8	85.3
Chloropicrin	74.6	49.1	76.0
Basamid/Sistan	59.5	50.1	76.1
Significance	0.011	NS (0.069)	0.032
LSD (15 df)	24.21	-	13.37

Table 14: Effect of pre-plant soil treatment on tree girth in 2007

Treatment	Circumference (mm) at 0.5 m above soil level		
	Acer	Sorbus	Tilia
Untreated	77.9	58.3	76.2
Sudan grass	87.7	57.8	84.8
BSD	90.9	64.6	89.9
Chloropicrin	86.7	68.3	83.8
Basamid/Sistan	93.7	69.6	84.2
Significance	NS (0.060)	0.030	NS (0.066)
LSD (15 df)	-	8.77	-

Table 15: Correlation of tree growth with level of *V. dahliae* in the soil

Tree growth	Correlation coefficients		
Parameter	2006	Log 2006	2007
Acer extension	-0.3308	-0.3157	-0.4988
Acer circumference	-0.4683	-0.4503	-0.4997
Sorbus extension	-0.5842	-0.6554	-0.3095
Sorbus circumference	-0.5723	-0.6378	-0.3543
Tilia extension	-0.4061	-0.2400	-0.3691
Tilia circumference	-0.5453	-0.3171	-0.3075

Levels of *V. dahliae* in soil - January 2008

The levels of *V. dahliae* recorded in all individual plots in January 2008 were greater than those recorded a year earlier (Table 16). The increase was greatest in the sudan grass plots, where levels in January 2008 were almost equal to those in untreated plots.

Levels of *V. dahliae* in January 2008 differed significantly between treatments (Table 17). Mean levels were significantly lower following soil treatment with BSD, Chlorofume or Basamid/Sistan 51 than in untreated soil. The two chemical treatments were particularly effective. The soil level of *V. dahliae* after sudan grass, did not differ significantly from the untreated control, in agreement with the result found in January 2006.

The reason for the increase in levels of *V. dahliae* between January 2007 and January 2008 is unknown. A possible explanation is release of the fungus from decaying roots of infected *Acer* and *Tilia* trees, or other decaying organic matter containing *V. dahliae*.

In January 2007, the level of *V. dahliae* in soil under an area of ryegrass adjacent to the experimental area was 30.8 cfu/g. This compares with a mean value of 25.9 for untreated plots. After one year under ryegrass, the level was unchanged, at 29.7 cfu/g. No broad-leaf weeds were found in the area when soil samples were collected in January 2008.

Table 16: Levels of *V. dahliae* in the soil – March 2005 to January 2008

Plot	Treatment	*V. dahliae* (cfu/g soil) Mar 05	Jan 06	Jan 07	Jan 08
1	Unt	7.0	28.0	31.7	55.6
2	Bas/Sis	11.7	0.5	1.1	3.5
3	Unt	9.3	7.6	16.4	38.9
4	Chl	9.3	0.1	1.0	17.4
5	Chl	11.3	0.6	1.7	6.5
6	Chl	4.9	0.6	0.1	1.9
7	Sud	9.5	7.3	18.0	36.4
8	Bas/Sis	7.5	0.4	0.1	0.8
9	BSD	10.8	7.5	1.9	37.1
10	Bas/Sis	10.7	0.0	0.0	0.3
11	Sud	8.8	24.0	4.5	65.2
12	Sud	10.9	30.4	7.3	38.2
13	BSD	8.0	9.9	3.0	31.2
14	BSD	14.0	4.3	3.7	18.0
15	Sud	31.4	36.4	14.8	54.9
16	Bas/Sis	19.5	0.2	0.0	2.1
17	Bas/Sis	20.6	0.7	4.2	4.3
18	Sud	16.8	17.1	10.6	21.0
19	Unt	17.8	62.2	20.4	47.6
20	BSD	38.2	26.1	15.7	43.6
21	Chl*	26.3	16.4	0.1	0.5
22	Unt	13.4	33.2	24.3	34.7
23	BSD	9.7	2.9	4.0	8.4
24	Chl	17.4	0.1	0.3	1.8
25	Unt	32.6	38.1	36.8	41.3
Ryegrass strip		-	-	30.8	29.7

*Treatment missed in autumn 2005; treated in May 2006.

Table 17: Effect of pre-plant soil treatments on mean levels of *V. dahliae* in soil as determined by conventional plate tests (cfu/g).

Treatment	Initial levels	Post-treatment levels		
	March 05	Jan 06	Jan 07	Jan 08
1. Untreated	16.0	33.8	25.9	43.6
2. Sudan grass	15.5	23.0	11.1	43.1
3. BSD	16.1	10.1	5.7	27.7
4. Chlorofume	14.3	2.5*	0.6	5.6
5. Basamid/Sistan 51	13.6	0.4	1.1	2.2
Significance	0.697	<0.001	<0.001	<0.001
LSD (15 df)	4.27	13.71	6.27	14.5

* Mean of 4 plots.

Soil levels of nematodes

Soils sampled on 30 May 2007 were found to contain low levels of stubby root nematodes (*Trichodorus* and *Paratrichodorus* species), stunt nematodes (*Tylenchorhynchus* and *Merlinius* species) and/or spiral nematodes (*Rotylenchus* and *Helicotylenchus* species). The levels of stubby root and stunt/spiral nematodes were significantly reduced in soils that had been treated with Chlorofume or Basamid/Sistan 51 in autumn 2005 (Table 18). No needle (*Longidorus* spp.) or dagger (*Xiphinema* spp.) nematodes were detected, and a few root lesion (*Pratylenchus* spp.) nematodes were found in just two plots (one sudan grass, one BSD)

Table 18: Effect of biological and chemical soil treatments on numbers of nematodes in soil – May 2007.

Soil treatment in 2005		No stubby root nematodes/L	No. stunt/spiral nematodes/L
1.	Untreated	255	85
2.	Sudan grass	215	90
3.	BSD	260	85
4.	Chlorofume	40	20
5.	Basamid/Sistan	45	20
Significance		0.004	0.003
SED	(15 df)	64.3	20.5

Quantifying *V. dahliae* in soil by a molecular test (PCR)

Results were erratic within samples, indicating inefficient extraction of nucleic acids. The EMR laboratory has now replaced the MoBio Power Soil DNA extraction with another, more effective protocol. Results were reasonably consistent within extractions, but disassociation analysis indicated an unidentified amplicon that disassociated at a higher temperature (approximately 88°C as opposed to the expected 81°C, obtained for the standards) as being responsible for most of the signal. Electrophoresis of the amplification products indicates that this product was larger than that derived from pure culture extracts of *V. dahliae.* Due to the uncertainty over interpretation of results, data are not presented here.

Conclusions

1. After declining by 23% between January 2006 and January 2007, the level of *V. dahliae* in untreated soil increased by 68% between January 2007 and January 2008. These results indicate release of *V. dahliae* propagules into the soil, possibly from decaying roots of infected *Acer* or *Tilia* trees.

2. After one year under a ryegrass sward, there was no change in the level of *V. dahliae* in the soil.

3. Definite symptoms of verticillium wilt first occurred in *Acer* and *Tilia* in the second growing season after planting in land infested with *V. dahliae*. No symptoms definitely associated with verticillium wilt occurred in *Sorbus*.

4. Pre-plant soil treatment with Basamid/Sistan or Chlorofume at the rates used significantly reduced infection of *Acer* and *Tilia* by *V. dahliae*. BSD appeared to reduce infection in *Tilia* but the difference was not statistically significant at p=0.05.

5. *Acer plantanoides* is more susceptible to infection by *V. dahliae* than *Tilia cordata*. A soil infestation of 0.1–0.7 cfu/g, as measured by the ADAS planting method, resulted in 10–12% infection in *Acer* and nil in *Tilia*.

6. The overall proportion of *Acer* trees found infected in 2007 (32%) was similar to that found infected in 2006 (36%).

7. Pre-plant soil treatment with BSD, sudan grass, Basamid/Sistan or Chlorofume, significantly increased mean extension growth of *Acer* and *Tilia*.

8. Pre-plant soil treatment with Basamid/Sistan or Chlorofume significantly increased stem circumference in *Sorbus.*

9. Basamid/Sistan or Chlorofume significantly reduced low levels of stubby root and stunt/spiral nematodes in the soil at 19 months after soil treatment; sudan grass and BSD were ineffective.

10. Attempts to quantify *V. dahliae* DNA in soil using a PCR method were unsuccessful.

Technology transfer

<u>Meeting</u>

- Project steering group meeting and site visit, 25 September 2007.

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Reference

Atallah, Z.K., Bae, J., Jansky, S.H., Rouse, D.I. & Stevenson, S.R. (2007). Multiplex real-time quantitative PCR to detect and quantify *Verticillium dahliae* colonisation in potato lines that differ in response to Verticillium wilt. *Phytopathology* **97:**865–872.

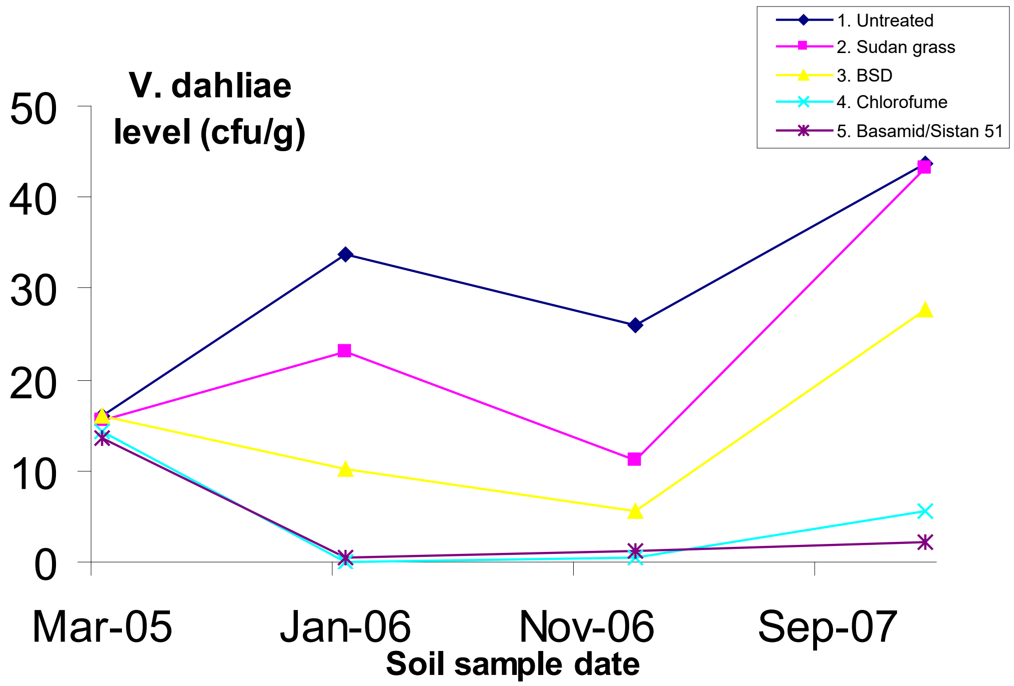
Appendix 1: Individual plot data – level of *V. dahliae* in soil and numbers of *Acer* and *Tilia* trees infected.

Treatment	Plot N°	Level of *V. dahliae* (cfu/g)		No. *Acer* trees (of 15) infected by *V. dahliae*		No. *Tilia* trees infected
		Mar 05	Jan 06	Oct 06	Oct 07	Oct 07
1. Untreated	1	7.0	28.0	0	9	1 (12)
	3	9.3	7.6	4	7 (13)	2
	22	13.4	33.2	5	6	2 (14)
	19	17.8	62.2	5	3	5 (14)
	25	32.6	38.1	11	0	3
2. Sudan grass	11	8.8	24.0	5	11	5
	7	9.5	7.3	7	5 (14)	0
	12	10.9	30.4	7	5	1
	18	16.8	17.1	4	4	1
	15	31.4	36.4	3	7	8 (14)
3. BSD	13	8.0	9.9	12	10	4
	23	9.7	2.9	2	4	0
	9	10.8	7.5	4	7	1
	14	14.0	4.3	7	8	0
	20	38.2	26.1	7	6	2
4. Chlorofume	6	4.9	0.6	7	4	0
	4	9.3	0.1	2	2	0 (14)
	5	11.3	0.6	0	3	0
	24	17.4	0.1	1	1 (14)	0
	21*	26.3	16.4	*	*	*
5. Basamid/Sistan 51	8	7.5	0.4	0	2	0
	10	10.7	0	0	3	0
	2	11.7	0.5	0	4 (14)	0
	16	19.5	0.2	0	1	0
	17	21.6	0.7	2	2	0

* Not planted with trees

( ) – number of trees assessed where less than 15

Appendix 2: Change in levels of *V. dahliae* in soil with time



Appendix 3. Crop diary: January 2007 - January 2008

30 May 2007 – Soil sampled from all plots to determine nematode numbers; trees inspected for verticillium wilt symptoms

11 September 2007 – Leaf yellowing assessed

8 October 2007 – Bark splitting assessed and trees sampled (one third removed)

13–14 November 2007 – Growth measured

14 January 2008 - Soil sampled to determine *V. dahliae* levels

February 2008 – Roots of sampled trees grubbed out