Project title:	Field grown trees: evaluation of chemical and biological pre-plant soil treatments for control of Verticillium wilt
Project number:	HNS 137
Report:	Final report, March 2010
Previous reports:	March 2006, March 2007, March 2008
Project leader:	Dr T M O'Neill ADAS Boxworth
Project consultant	Dr T Locke
Key workers:	Miss S Wynn, Dr M Selby, Mr D Smith, Ms A Huckle and Miss A Shepherd, ADAS Mr J Adlam, Dove Associates Dr D Yohalem, EMR
Location of project:	ADAS Arthur Rickwood, ADAS Boxworth Andlers Ash Nursery, Liss, Hants
Project co-ordinators:	Mr H Arshadi Mr J Adlam
Date project commenced:	1 April 2005
Date completion due:	31 March 2010
Key words:	*Acer*, *Tilia*, *Sorbus*, *Verticillium dahliae,* wilt, soil treatment, survival, Biological Soil Disinfestation, sudan grass, chloropicrin, Basamid, dazomet, metam sodium, tree, methyl bromide, nematode, growth

Disclaimer

Whilst reports issued under the auspices of the HDC are prepared from the best available information, neither the authors nor the HDC can accept any responsibility for inaccuracy or liability for loss, damage or injury from the application of any concept or procedure discussed.

The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of results.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use except where the crop or situation is the subject of an off-label extension of use.

Before using all pesticides and herbicides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

Further information

If you would like a copy of the full report, please email the HDC office (hdc@hdc.org.uk), quoting your HDC number, alternatively contact the HDC at the address below.

Horticultural Development Company
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel: 0247 669 2051
Fax: 0247 647 8905

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.

All information provided to the HDC by ADAS in this report is provided in good faith. As ADAS shall have no control over the use made of such information by the HDC (or any third party who receives information from the HDC) ADAS accept no responsibility for any such use (except to the extent that ADAS can be shown to have been negligent in supplying such information) and the HDC shall indemnify ADAS against any and all claims arising out of use made by the HDC of such information.

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.

Dr T M O'Neill
Principal Research Scientist
ADAS Boxworth

Signature .... Date ....

Report authorised by:

Mr J Clarke
Science and Business Development Manager
ADAS Boxworth

Signature .... Date ....

CONTENTS

GROWER SUMMARY

Headline

- Verticillium wilt was significantly reduced in *Acer* by pre-plant soil treatment with Chlorofume or a combined Basamid/Sistan 51 treatment, and in *Tilia* by these treatments and Biological Soil Disinfestation. A sudan grass treatment was found to be ineffective.

- The risk of Verticillium wilt infection in *Tilia* increased greatly above 10 colony forming units (cfu) per g of soil of *V. dahliae* whereas the risk in *Acer* was high where even low (0.1-1.0 cfu/g) levels of the fungus were detected pre-planting.

Background and expected deliverables

In 2009 the area of field-grown ornamental and amenity trees in England was estimated to be around 1,000 ha, valued at £19.8 million. 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.

From 1 January 2007, methyl bromide was no longer permitted for pre-plant soil disinfestation for tree production. 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 the project was to identify one or more alternatives to methyl bromide for soil disinfestation for field-grown trees. 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 included:

- 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 these soil treatments on nematode populations, initial weed control and 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

Soil disinfestation treatments

An experiment was established in 2005, on land naturally infested with *V. dahliae*, to determine the effectiveness of five soil treatments:

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 2005 as a green manure. No soil cover.

3. Biological Soil Disinfestation (BSD). Italian rye-grass (cv. Danergo) incorporated on 9 August 2005 (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 for 3 months 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 rate) by contractor on 26 August 2005.

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 rate) by contractor on 26 August 2005 (Figure 1).

Full details of treatment applications and soil conditions at the time of treatment are provided in the March 2006 Annual Report.

Soil levels of *V. dahliae* and nematodes were determined in March 2005 before soil treatment. Levels of *V. dahliae* ranged from 4.9 to 38.2 colony forming units (cfu) per g of soil with a mean of 15.1 cfu/g. Levels of nematodes were low (<300/litre).

a) Trial view with Sudan grass plots clearly visible

b) Incorporation of ryegrass for biological soil disinfestation

c) Sealing of ryegrass plots

d) Basamid application and metam-sodium injection pre-planting

e) Chloropicrin injection pre-planting with sealing of soil

f) Basamid treated area after smear seal and before covering

Figure 1: Application of soil disinfestation treatments in large replicated plots - 2005

Reduction of V. dahliae levels in soil

The two chemical treatments were particularly effective in reducing mean levels from 13.6 – 16.0 cfu/g (March 2005) to 0.4 – 2.5 cfu/g (January 2006) (Figure 2). Three years later (December 2008) the levels in these treatments were still relatively low at 1.5 – 5.6 cfu/g. Over this period the levels in untreated soils planted with *Acer* and

Tilia increased to 50 cfu/g. The BSD treatment also significantly reduced levels of *V. dahliae* in the soil, though less effectively than the chemical treatments. Sudan grass had no significant effect on levels of the fungus. Increases in mean levels of *V. dahliae* over time may be due to the release of the fungus from decaying infected roots.

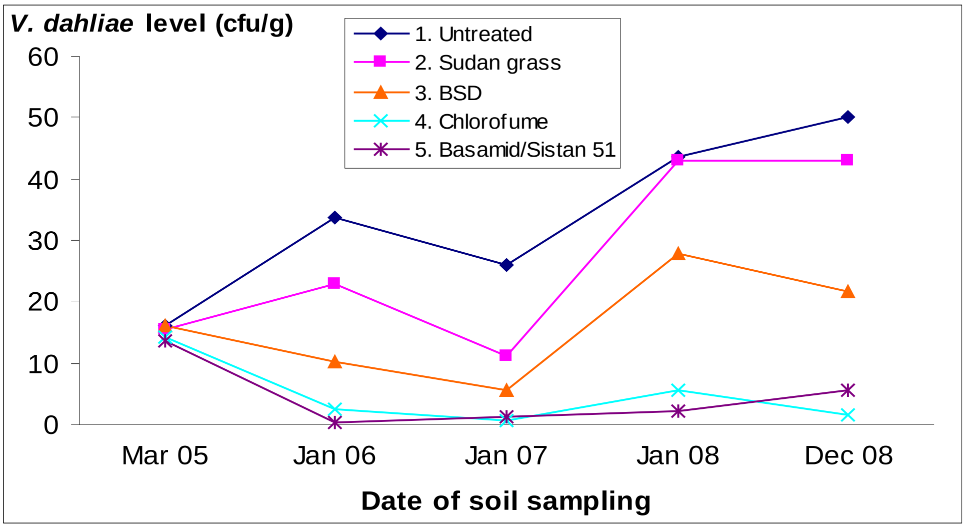

Figure 2: Effect of soil treatment in autumn 2005 on the level of *V. dahliae* in soil (cfu/g) and subsequent changes with time

Occurrence of Verticillium wilt symptoms

Acer platanoides 'Emerald Queen', *Tilia cordata* 'Greenspire' and *Sorbus aria* 'Majestica' on *Sorbus intermedia* rootstock were planted in April 2006 (Figure 3). Symptoms of Verticillium wilt were first seen in the second growing season (2007). The incidence of leaf yellowing and bark splitting in *Acer*, typical symptoms of Verticillium wilt in this species, was reduced by the two chemical treatments (Table 1). After three growing seasons the incidence of bark splitting in *Acer* had generally increased but was still reduced by the two chemical treatments (Table 1). There was no significant treatment effect on leaf yellowing in *Sorbus* or *Tilia.*

Recovery of V. dahliae from trees

A sample of 50 trees of each species was tested for *V. dahliae* prior to planting. None was found in *Tilia* or *Sorbus* and a low level (2%) in *Acer*. Given the high level of infection found in *Acer* at 6 months after planting and the large differences in levels of infection between trees in untreated and treated soil, it is very probable that infection in trees mostly originated from the soil.

a) Trial view in first year after planting – 2006

b) Comparison of growth after Basamid / metam-sodium treatment (left) with untreated (right) in year 3 - 2008

c) Branch die-back in *Acer*

d) Patch of dying trees due to Verticillium wilt

e) Bark-split and stained wood in *Acer* indicative of Verticillium wilt

f) Growth of *V. dahliae* from an *Acer* stem base section in laboratory test

Figure 3: Tree growth in the field experiment (2006 – 2008) and symptoms of Verticillium

Table 1: Effect of pre-plant soil treatment on symptoms of Verticillium wilt in *Acer*

Treatment	Mean % trees affected by:			
	Bark splitting		Leaf yellowing	
	2007	2008	2007	2008
1. Untreated	22.1	39.6	33.9	9.1
2. Sudan grass	23.3	37.9	31.2	1.3
3. BSD	19.1	32.9	24.6	1.1
4. Chlorofume	7.9	17.9	12.1	2.0
5. Basamid/Sistan 51	6.1	4.1	6.7	1.2

Bark splitting ≥ index 2; leaf yellowing ≥ index 2; no symptoms occurred in 2006.

In October 2006, one third of the trees in each plot were cut down and the stem bases 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 significantly fewer trees grown following soil treatment with Basamid/Sistan 51 (3%) or Chlorofume (17%). Neither Sudan grass nor BSD reduced the incidence of infection. No infection was found in *Tilia* or *Sorbus*.

In October 2007 a further third of the Acer trees were cut down and tested for infection in the stem base. *V. dahliae* was recovered from 35% of trees grown in untreated soil and from significantly fewer following soil treatment with Basamid/Sistan 51 (17%) or Chlorofume (16%). The same two treatments reduced the incidence of infection in *Tilia* from 18% to 0% (Table 2). No *V. dahliae* was recovered from *Sorbus*.

Table 2: Effect of pre-plant soil treatment on recovery of *V. dahliae* from *Acer* and *Tilia* stem bases after one, two and three years' growth on infested land

Treatment	Mean % trees infected at stem base:					
	Acer			*Tilia*		
	2006	2007	2008	2006	2007	2008
1. Untreated	33.3	34.7	NT	0	18.1	31.5
2. Sudan grass	34.6	43.7	NT	0	19.7	17.6
3. BSD	42.7	47.2	NT	0	8.9	11.7
4. Chlorofume	16.7	15.6	NT	0	0.0	2.9
5. Basamid/Sistan 51	2.6	16.5	NT	0	0.0	11.7

NT – Not tested

In December 2008, only the remaining third of *Tilia* trees was tested for *V. dahliae.* The fungus was recovered from 32% of trees grown in untreated soil and from 3-18% of trees following soil treatment, Chlorofume being the most effective (Table 2).

Soil level of V. dahliae and risk of Verticillium wilt

In autumn 2007 and 2008, the incidence of Verticillium wilt symptoms in *Acer* (leaf yellowing and bark cracking) increased with soil level of *V. dahliae* (Table 3). *Tilia* leaf symptoms were slight and there was no marked relationship between leaf yellowing and soil level of *V. dahliae* at planting.

Table 3: Occurrence of Verticillium wilt symptoms in *Acer* and *Tilia* in autumn 2007 and 2008 according to three soil levels of *V. dahliae* at planting in 2006

V. dahliae in soil (cfu/g)* in 2006	No. of plots	*Acer* leaf yellowing	*Acer* bark splitting	*Tilia* leaf yellowing
	2007			
<0.7	9	8.8	6.9	0
2.9 – 10.0	6	26.4	17.9	0.6
>10.0	9	32.3	23.9	4.1
	2008			
<0.7	9	1.5	10.1	7.6
2.9 – 10.0	6	7.8	34.0	13.3
>10.0	9	6.9	38.8	11.4

*There were no soils with *V. dahliae* levels between 0.7 and 2.9 cfu/g. The limit of detection is 0.1 cfu/g.

The incidence of *Tilia* trees confirmed as infected by *V. dahliae*, irrespective of whether or not leaf symptoms occurred, showed a moderately good statistical correlation with soil inoculum levels of the fungus at planting. In contrast, the incidence of *Acer* trees confirmed as infected did not show a good correlation, with soil levels of *V. dahliae* accounting for 38% of the variance. This is consistent with the greater susceptibility to Verticillium wilt shown by *Acer*, where even a very low level of *V. dahliae* in the soil can result in a high incidence of infected trees. The incidence of infected *Acer* trees was notably greater above 2.9 cfu/g, whereas the incidence of infected *Tilia* trees was notably greater only above 10.0 cfu/g (Table 4).

Table 4: Infection of *Acer* and *Tilia* stem bases according to three soil levels of the fungus at planting

V. dahliae in soil (cfu/g) at planting * in 2006	Mean % trees infected at stem base (number of years crop planted) see below:			
	Acer		*Tilia*	
	2006 (1 growing season)	2007 (2 growing seasons)	2007 (2 growing seasons)	2008 (3 growing seasons)
1. <0.7 (n=9)	8.9	12.8	0	7.8
2. 2.9 – 10.0 (n=6)	38.9	43.6	6.7	10.0
3. > 10.0 (n=9)	34.8	36.3	21.7	27.8

* There were no soils with *V. dahliae* levels between 0.9 and 2.9 cfu/g.

Effect of soil treatments on tree growth

The extension growth of *Acer* and *Tilia* in 2007 and circumference in 2008 was significantly increased by all four soil treatments. There was a trend for reduced growth with increasing soil levels of *V. dahliae* at planting but the statistical correlations were poor. Chlorofume and Basamid/Sistan 51 significantly increased stem circumference of *Sorbus* in both years, whereas the two biological treatments had no effect. The growth response in *Sorbus* to the chemical 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.

Progression of tree death with time

The cumulative proportion of *Acer* trees that had died after three years (5.3%) was greater than that of *Tilia* (3.0%) or *Sorbus* (2.4%) very probably due to the greater incidence of Verticillium wilt in *Acer*. Death of *Acer* trees mostly occurred in the third year. The occurrence of dead *Tilia* trees increased steadily with time, consistent with an increasing occurrence of field symptoms of Verticillium wilt of *V. dahliae* and increasing recovery from the tree stem bases with time. Most of the *Sorbus* tree death occurred in the first year and was primarily due to poor establishment in the dry summer of 2006

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 51, but were not significantly reduced by the two biological treatments.

Effect of tree removal and soil cultivation on V. dahliae levels in soil

The effect of grubbing trees infected by Verticillium wilt on subsequent levels of *V. dahliae* in the soil is unknown. With potato crops affected by Verticillium wilt, there can be a large (e.g. fivefold) increase in *V. dahliae* levels in the soil in the 12 months after harvest, probably arising as infested potato haulm rots down and releases microsclerotia into the soil. In this project, all trees were grubbed in April 2009. The mean level of *V. dahliae* pre-grubbing was 24.3 cfu/g, and 9 months after grubbing (December 2009) this was virtually unchanged (22.8 cfu/g). This lack of increase is probably due to the slower decay of tree roots compared with potato haulm.

Effect of ryegrass on V. dahliae

For control of *Verticlllium albo-atrum* in hops, the pathogen level in soil can be reduced markedly by two or more years of weed-free grass prior to planting. In a strip or ryegrass adjacent to the field experiment, the level of *V. dahliae* under a largely weed-free ryegrass sward remained constant for 2 years at around 31 – 36 cfu/g and after 3 years had declined to 14 cfu/g. The potential for using a ryegrass sward to reduce *V. dahliae* in soil appears to be limited.

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

Initial soil samples from the field experiment were tested for *V. dahliae* DNA using a TaqMan PCR test at EMR. 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*.

Financial benefits

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.

Action points for growers

1. Do not plant *Acer platanoides* on land where any *V. dahliae* has been detected. After just 6 months on land with 0.1–0.7 cfu/g, 10% of trees had become infected.

2. Do not plant *Tilia cordata* on land with soil levels above 0.7 cfu/g if trees are to be grown for more than 2 years. Although *Tilia* can tolerate a higher soil infestation of *V. dahliae* than *Acer platanoides*, there is a risk of some infection in the third season, even at low levels of *V. dahliae*. If no land free of *V. dahliae* is available, and a low level of Verticillium wilt can be tolerated, consider planting on soils with up to 10 cfu/g.

3. Of the treatments examined, Chlorofume or Basamid/Sistan 51, at the rates used in this experiment were the best pre-plant soil treatments. They consistently reduced soil *V. dahliae* by at least 90% to 0.1-0.7 cfu/g, prevented infection of *Tilia cordata* by *V. dahliae* in the first 18 months after planting and significantly reduced infection in *Acer*. However, results suggest this degree of reduction is insufficient to prevent substantial infection of *Acer* if *V. dahliae* is still detected in the soil after treatment.

4. BSD, applied as described in this report, may be suitable for less susceptible species and low levels of *V. dahliae*. It significantly reduced infection of *Tilia cordata.*

5. Be aware that many dicotyledonous weeds that are common in the UK, including fat hen (*Chenopodium alba)* and shepherd's purse (*Capsella bursa-pastoris*), and several crops commonly grown in the UK, can act as hosts of *V. dahliae*. A list of crop and weed species that have been found in the UK to host *V. dahliae* is given in the Year 2 report (March 2007).

6. **It is strongly recommended** that a pre-plant soil test, to determine the soil levels of *V. dahliae,* is conducted prior to planting a highly susceptible crop, such as *Acer,* even if there is no recent past history of a susceptible crop having been grown on the land.

SCIENCE SECTION

Introduction

In the first stage of this experiment, two chemical soil disinfestation treatments 'Basamid' (98% dazomet) / 'Sistan 51' (51% metam sodium) and 'Chlorofume' (99.5% chloropicrin) and one biological treatment (Biological Soil Disinfestation with Italian rye-grass (cv. Danergo)) significantly reduced the levels of *V. dahliae* in soil; a second biological treatment (sudan grass (cv. Nigrum)) 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 was to determine how two Verticillium-susceptible tree species (*Acer* and *Tilia*) and one resistant species (*Sorbus*) respond to different levels of *V. dahliae* in the soil. Results were examined, both with regard to the original soil treatment and with regard to soil levels of *V. dahliae* irrespective of treatments.

Definite symptoms of Verticillium wilt first occurred in *Acer* and *Tilia* in the second growing season (2007). Pre-plant soil treatment with Basamid/Sistan 51 or Chlorofume at the rates used significantly reduced infection of both species by *V. dahliae*, as determined by recovery of the fungus from stem bases. BSD also appeared to reduce infection in *Tilia* in 2007 but the difference was not statistically significant at P=0.05. No *V. dahliae* was recovered from the *Sorbus* stem bases tested.

The objectives in the final stage of the project (April 2008 - March 2010) were:

- To determine the incidence of Verticillium wilt symptoms in *Acer*, *Tilia* and *Sorbus* after three growing seasons;
- To determine the incidence of *V. dahliae* infection in the base of *Tilia* trees after three growing seasons;
- To determine if there is any basis for developing strategic planting of two different tree species (*Acer* and *Tilia*) based on levels of *V. dahliae* in the soil;
- To determine the effect of soil treatments applied in 2005 on tree growth up to autumn 2008;
- To monitor levels of *V. dahliae* in soil at three years after soil treatment;

- To monitor levels of *V dahliae* in soil after two and three years under a ryegrass sward adjacent to the trial site;
- To determine the effect of soil disturbance caused by grubbing out infected trees and soil cultivation on the level of *V. dahliae* in the trial site soil nine months later.

Materials and methods

Field assessments:

All trees were assessed on 7 August 2008 to determine the occurrence of leaf yellowing and necrosis in all three species, and on 19 November 2008 to determine bark splitting on the main stem of *Acer*, a symptom typical of Verticillium wilt. The occurrence of dead trees and of trees with dead tops were recorded at the first assessment and also considered as 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 – 3 scale; trees with a score of 2 or 3 were considered to be showing symptoms of Verticillium wilt. The categories were:

0 – Nil
1 – Bubbling and small splits (1-2 cm long; <1 mm wide)
2 – Definite cracking of bark (>2 cm long; >1 mm wide)
3 – Severe cracking of bark (>10 cm long; >2 mm wide)

Tree growth was determined on 19-20 November 2008 by measuring the extension growth of the main leader on 15 trees of each species per plot (the central plant in each group of the three originally planted). The trunk circumference at 50 cm above soil level was measured on the same trees.

The remaining central *Tilia* tree in each group of three was cut off at ground level. A 30 cm length of stem was removed from the stem base, at around 20 cm height (above the graft), from the first 10 live plants in each plot for laboratory testing for the presence of *V. dahliae* in the tissues.

Laboratory tests:

Stem bases were tested for *V. dahliae* as described previously (see Year 2 Annual Report). Stem bases were also assessed for staining of wood, as described previously.

Soil samples were taken from each plot on 11 December 2008 and tested for *V. dahliae*. The trial area was subsequently divided into three broadly equal areas and the corners marked by burying magnets at 35 cm depth. After removal of tree roots in spring 2009 and soil cultivation, the corners were re-found using a magnet detector and marked with canes. Soil samples were collected from the three areas in December 2009 and tested for *V. dahliae*. Samples were collected and tested as previously described.

Statistical analysis:

Results were examined by ANOVA or by regression analysis using the logit link function where data were binomially distributed. The relationship of Verticillium wilt symptoms and infection of trees by *V. dahliae* to soil levels of *V. dahliae* was not examined statistically for individual assessments due to the limited data set. Data on infection of trees were combined over years and examined by regression analysis.

Results and discussion

<u>Field symptoms of Verticillium wilt – autumn 2008</u>

Acer:

In August 2008, presumptive Verticillium wilt was found in around 35% of trees on untreated soil and significantly less (7 – 12%) following the two chemical soil treatments (Table 5). Tree death and leaf yellowing as individual categories appeared to be reduced by the two chemical soil treatments but differences were not quite statistically significant at P=0.05. Soil treatment had no significant effect on the incidence of trees with dead tops or on leaf yellowing severity (data not shown). However, the occurrence of leaf yellowing and the combined Verticillium wilt symptom category were related to the level of *V. dahliae* in the soil at planting (Table 6) and to levels in January 2008 (Table 7).

In November 2008, both the incidence and severity of bark splitting in *Acer* were reduced by the two chemical treatments and not by the other treatments (Table 8). The incidence and severity of bark cracking *Acer* at this time appeared to be related to the level of *V. dahliae* in the soil at planting (Table 9). The two chemical treatments, but not the biological treatments, had greatly reduced *V. dahliae* levels in soil pre-planting.

Table 5: Effect of pre-plant soil treatment on occurrence of dead trees and leaf yellowing and/or necrosis of *Acer* – field assessment, August 2008

Treatment	Soil *V. dahliae* levels, Jan 06 (cfu/g)	Mean % trees		
		Dead	Leaf yellowing	Dead, dead top, yellowing, bark split
1. Untreated	33.8	15.7 (5.2)	9.1 (3.6)	35.4 (8.5)
2. Sudan grass	23.0	14.9 (5.1)	1.3 (1.5)	28.4 (8.1)
3. BSD	10.1	15.5 (5.3)	1.1 (4.1)	36.5 (8.8)
4. Chlorofume	2.5	1.9 (2.4)	2.0 (2.2)	11.8 (7.0)
5. Basamid/Sistan 51	0.4	2.6 (2.3)	1.2 (1.4)	6.5 (4.3)
Significance (15 df)	<0.001	0.063	0.068	0.035

Split bark: index 2 or 3; Leaf yellowing: index 3 or 4. ( ) – standard error.

Table 6: Occurrence of Verticillium wilt symptoms in *Acer* according to soil levels of *V. dahliae* prior to planting (January 2006) – field assessment, August 2008

Level of *V. dahliae* (cfu/g) in January 2006*	Number of plots	Mean % *Acer* trees with	
		Leaf yellowing	Dead, dead top, yellowing, bark split
<0.7	9	1.5	8.5
2.9–10.0	6	7.8	29.1
>10.0	9	6.9	37.5

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

Table 7: Occurrence of Verticillium wilt symptoms in *Acer* according to soil levels of *V. dahliae* in January 2008 – field assessment, August 2008

Levels of *V. dahliae* (cfu/g) in January 2008	Number of plots	Mean % *Acer* trees with		
		Leaf yellowing	Dead top	Dead, dead top, yellowing, bark split
<0.1-1	2	0	3.3	3.3
2-10	7	2.9	1.9	8.7
11-40	9	6.0	12.2	28.8
>40	6	8.1	13.5	43.6

Table 8: Effect of pre-plant soil treatment on occurrence of bark splitting of *Acer* – field assessment, November 2008

Treatment	Soil *V. dahliae* levels, Jan 06 (cfu/g)	% trees affected (index 2 or more)	Mean severity (index 0-3)
1. Untreated	33.8	39.6 (7.7)	1.1
2. Sudan grass	23.0	37.9 (8.1)	1.1
3. BSD	10.1	32.9 (8.2)	0.9
4. Chlorofume	2.5	17.9 (7.1)	0.5
5. Basamid/Sistan 51	0.4	4.1 (3.1)	0.2
Significance (15 df)		0.006	0.007
LSD	<0.001	-	0.51

Split bark: index 2 or 3; ( ) – standard error.

Table 9: Effect of soil level of *V. dahliae* at planting on occurrence of bark cracking in *Acer* – November 2008

V.dahliae in soil (cfu/g)	Number of plots	% trees affected	Mean severity (0-3)
<0.7	9	10.1	0.34
2.9 – 10	6	34.0	0.96
< 10.0	9	38.8	1.10

Tilia:

In August 2008, presumptive Verticillium wilt was found in around 15% of trees in untreated soil plots. Pre-planting soil treatment had no significant effect (P >0.05) on leaf yellowing incidence (Table 10) or severity (data not shown). The incidence of dead trees was very low, except on soil treated with sudan grass. The occurrence of trees with any suspect Verticillium wilt symptom increased with the pre-planting soil level of *V. dahliae* (Table 11).

Table 10: Effect of pre-planting soil treatment on occurrence of leaf yellowing and dead trees in *Tilia* – field assessment, August 2008

Treatment	Soil *V.dahliae* Levels, Jan 06 (cfu/g)	Mean % trees		
		Dead	Leaf yellowing	Dead, leaf yellowing or dead top
1. Untreated	33.8	0	15.1 (7.7)	19.1 (8.0)
2. Sudan grass	23.0	10.3	8.3 (5.9)	19.0 (8.0)
3. BSD	10.1	0	13.5 (7.3)	14.8 (7.1)
4. Chlorofume	2.5	0	5.6 (5.8)	11.4 (7.7)
5. Basamid/Sistan 51	0.4	1.3	9.5 (6.3)	10.9 (6.4)
Significance (15 df)	<0.001	<0.001	0.867	0.890

( ) – standard error

Table 11: Occurrence of Verticillium wilt symptoms in *Tilia* according to soil levels of *V. dahliae* at planting – field assessment, August 2008

Levels of *V. dahliae* (cfu/g) in January 2006	Mean % trees			
	Number of plots	Dead	Leaf yellowing	Dead, dead top or leaf yellowing
<0.7	9	0.7	7.6	10.9
2.9-10	6	0	13.3	15.6
>10	9	6.1	11.4	19.2

Sorbus:

In August 2008, occurrence of leaf yellowing and dead trees was very low. The dead trees occurred on soil treated with BSD or Chlorofume pre-planting, and not on untreated soil (Table 12). These results indicate that *V. dahliae* was not the cause of death of *Sorbus* trees.

Table 12: Effect of pre-planting soil treatments on occurrence of leaf yellowing and dead trees in *Sorbus* – field assessment, August 2008

Treatment	Soil *V.dahliae* levels, Jan 06 (cfu/g)	Mean % trees		
		Dead	Leaf yellowing	Dead, leaf yellowing or dead top
1. Untreated	33.8	0	0	0
2. Sudan grass	23.0	0	0	0
3. BSD	10.1	4.2 (1.3)	0	4.2 (1.6)
4. Chlorofume	2.5	1.4 (0.7)	0	1.4 (0.9)
5. Basamid/Sistan 51	0.4	0	1.3	1.4 (0.9)
Significance (15 df)	<0.001	0.002	-	0.03

( ) - standard error

<u>Recovery of *V. dahliae* from *Tilia* trees in 2008</u>

Overall, *V. dahliae* was isolated from the stem bases of 16 % of *Tilia* trees tested in 2008. Given that no *V. dahliae* was found in a sample of *Tilia* trees tested pre-planting (see Year 2 Annual 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.

Infection of *Tilia* by *V. dahliae* as determined in 2008 was greatest on untreated soil (32% trees infected). It appeared to be reduced by pre-plant soil treatment with Chlorofume, Basamid/Sistan 51 or BSD but differences were not significant at

P<0.05 (Table 13). When the data from 2007 and 2008 were combined to give a larger data set for analysis, Basamid/Sistan 51, Chlorofume and BSD all significantly reduced infection of *Tilia* by *V. dahliae* (P=0.007).

Table 13: Effect of pre-plant soil treatment on recovery of *V. dahliae* from *Tilia* stem bases

Treatment	Soil *V. dahliae* levels, Jan 06 (cfu/g)	Mean % trees *V. dahliae* isolated		
		2007	2008	2007 + 2008
1. Untreated	33.8	18.1 (5.1)	31.5 (7.8)	24.2 (5.9)
2. Sudan grass	23.0	19.7 (5.1)	17.6 (6.4)	19.4 (5.4)
3. BSD	10.1	8.9 (3.7)	11.7 (5.4)	10.4 (4.2)
4. Chlorofume	2.5	0.0 (0)	2.9 (3.4)	1.0 (1.5)
5. Basamid/Sistan 51	0.4	0.0 (0)	11.7 (5.4)	4.8 (2.9)
Significance (15 df)	<0.001	0.002	0.076	0.007

( ) standard error

<u>Wood staining</u>

Wood staining was commonly found both within *Tilia* stems and below the bark (Table 14). The association of *V. dahliae* recovery from stained and unstained wood was examined. Overall, *V. dahliae* was recovered from 16% of stem bases with stained wood and from 13% of stem bases with unstained wood. There were no significant differences between soil treatments (Table 15).

Table 14: Occurrence of wood staining in *Tilia* stem bases – 2008

Treatment	Soil *V. dahliae* levels, Jan 06 (cfu/g)	Mean % stems with definite wood staining	
		Internal	External
1. Untreated	33.8	70.0 (9.2)	36.3 (11.1)
2. Sudan grass	23.0	76.0 (8.5)	36.3 (11.1)
3. BSD	10.1	66.0 (9.4)	38.3 (11.2)
4. Chlorofume	2.5	54.2 (11.5)	28.8 (11.6)
5. Basamid/Sistan 51	0.4	46.2 (10.0)	24.2 (9.8)
Significance (15 df)	<0.001	NS	NS

( ) standard error

Table 15: Association of wood staining and isolation of *V. dahliae* from *Tilia* – 2008

Treatment		Soil *V. dahliae* levels, Jan 06 (cfu/g)	% stems from which *V. dahliae* recovered	
			Stained wood	Unstained wood
1.	Untreated	33.8	33.9 (9.4)	22.3 (9.3)
2.	Sudan grass	23.0	18.2 (7.4)	17.3 (10.9)
3.	BSD	10.1	8.8 (5.7)	12.7 (8.2)
4.	Chlorofume	2.5	4.8 (5.6)	0.0 (0)
5.	Basamid/Sistan 51	0.4	11.9 (7.6)	11.0 (5.7)
Mean			15.5	12.6
Significance (15 df)		<0.0001	0.141	0.278

( ) standard error

<u>Association of field symptoms with isolation of *V. dahliae* from *Tilia* - 2008</u>

Recovery of *V. dahliae* from the stem bases of *Tilia* trees was not closely related to the occurrence of field symptoms of verticillium wilt (leaf yellow index >2) (Table 16). *V. dahliae* was recovered from 7/23 (30%) of trees with field symptoms and from 30/217 (14%) of those without symptoms. Possibly some of the leaf yellowing scored as presumptive Verticillium wilt was due to some other cause, or to infection occurring in the trees with leaf yellowing below the level at which the stem base sections were taken for the isolation test.

Table 16: Association of presumptive Verticillium wilt symptoms in *Tilia* with recovery of *V. dahliae* from the stem base of these trees - 2008

	Number of trees in each category	
	Leaf yellowing	No symptoms
V. dahliae isolated	7	30
V. dahliae not isolated	16	187
Total	23	217

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

The effect of the level of *V. dahliae* in the soil at planting on the incidence of *Tilia* trees that became infected with the fungus was examined (Figure 4). When the results for stem base isolation in 2007 and 2008 are combined to create a larger data set, the incidence of tree infection was around three to eight times greater at soil infestation levels of more than 10.0 cfu/g, when compared with soil levels less than 10.0 cfu/g (Table 17). A regression of log10 soil infestation level in January 2006, just before planting, against the total proportion of *Tilia* trees infected, was significant ($P<0.001$) and accounted for 57% of the variance. This compares with soil infestation levels accounting for 38% of variance in *Acer* tree infection (combined 2006+2007 isolation data). The greater correlation of soil *V. dahliae* levels with incidence of infection in *Tilia* than in *Acer* is consistent with the high susceptibility of *Acer*, where even very low levels of *V. dahliae* in the soil can result in a high incidence of infection.

In contrast to the isolation results of 2007, in 2008 *V. dahliae* was isolated from *Tilia* trees grown in soil with 0.7 cfu/g or less. These results indicate that *T. cordata* is susceptible to *V. dahliae* even at very low soil levels where trees are grown in a soil for at least three years, although the risk of infection appears much less than that of *A. platanoides*.

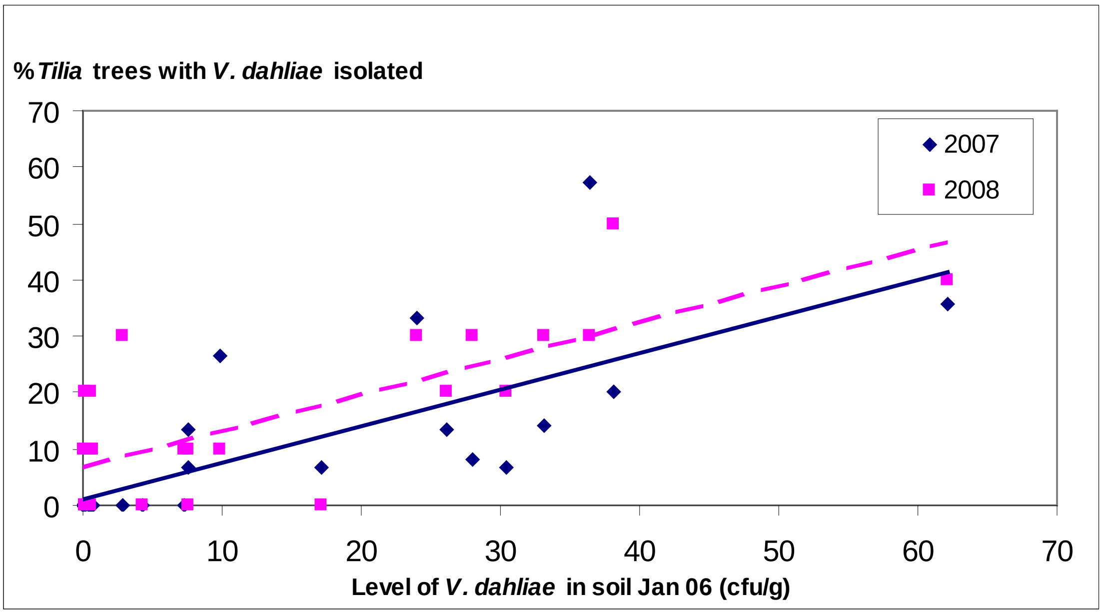

Figure 4. Effect of level of soil infestation with *V. dahliae* at planting on recovery of the fungus from stem bases of *Tilia* after two and three years.

Table 17: Recovery of *V. dahliae* from *Tilia* trees in autumn 2008 grouped according to three levels of soil infestation at planting

*V. d*ahliae level (cfu/g) in soil – Jan 06*	No. plots in category	Mean % trees infected/plot 2008	Mean % trees infected/plot 2007 + 2008
<0.7	9	7.8	3.1
2.9 – 10.0	6	10.0	8.7
>10.0	9	27.8	24.3

*There were no soils with *V. dahliae* levels between 0.9 and 2.7 cfu/g. 10 trees / plot in 2008; 25 trees / plot for 2007 + 2008.

Effect of soil treatment on tree growth

By autumn 2008, all four soil treatments had significantly increased the circumference of *Acer* and *Tilia* trees (Table 18). The circumference of *Sorbus* trees was increased by the two chemical treatments and not by the two biological treatments. It is possible that the increased circumference in *Acer* and *Tilia* was due at least in part to control of infection by *V. dahliae* in these species.

Soil treatment did not significantly increase extension growth of *Acer* or *Sorbus* compared with untreated plots in 2008 (Table 19). The extension growth of *Tilia* was increased significantly only by the BSD treatment.

In addition to effects on soil *V. dahliae* levels, pre-plant soil treatments may also have influenced growth through effects on soil microorganisms and nutrients.

Table 18: Effect of pre-plant soil treatment on tree girth in 2008

Treatment	Soil *V. dahliae* levels, Jan 06 (cfu/g)	Circumference (cm) at 0.5 m above soil level		
		Acer	*Sorbus*	*Tilia*
Untreated	33.8	9.2	7.2	10.3
Sudan grass	23.0	11.1	6.6	11.8
BSD	10.1	11.2	7.9	12.6
Chlorofume	2.5	11.5	8.4	11.8
Basamid/Sistan 51	0.4	11.9	9.5	11.8
Significance	<0.001	0.018	<0.001	0.038
LSD (15 df)		1.52	1.00	1.34

Table 19: Effect of pre-plant soil treatment on extension growth in 2008

Treatment	Soil *V. dahliae* levels, Jan 06 (cfu/g)	Extension growth (cm)		
		Acer	*Sorbus*	*Tilia*
Untreated	33.8	19.6	35.0	31.6
Sudan grass	23.0	25.8	28.4	35.6
BSD	10.1	23.6	41.3	34.9
Chlorofume	2.5	25.1	35.6	36.6
Basamid/Sistan 51	0.4	25.1	39.6	38.5
Significance		0.287	0.012	0.376
LSD (15 df)	<0.001	6.42	6.98	7.20

Effect of soil infestation with *V. dahliae* on tree growth

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 December 2008. 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 20). The strength of the correlations was not consistently 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.

No symptoms of Verticillium wilt were observed in *Sorbus* in the experiment. 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, and the two chemical treatments reduced this disease.

Table 20: Correlation of tree growth measured in 2008 with level of *V. dahliae* in the soil in 2006 and 2008

Tree growth	Correlation coefficients		
Parameter	2006	Log 2006	2008
Acer extension	-0.4577	-0.3395	-0.0663
Acer circumference	-0.5940	-0.5557	-0.4225
Sorbus extension	-0.2526	-0.2978	-0.3576
Sorbus circumference	-0.5335	-0.6870	-0.6923
Tilia extension	-0.4292	-0.3538	-0.2279
Tilia circumference	-0.4763	-0.2785	-0.2045

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

The levels of *V. dahliae* recorded in all individual plots in December 2008 were generally similar to those recorded a year earlier (Table 21). Mean levels recorded each year since 2005 are shown in Figure 1 in the Grower Section of this report.

Levels of *V. dahliae* in December 2008, as in previous years, differed significantly between treatments (Table 22). 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 results of previous years.

It has been reported that soil infestation levels of *V. albo-atrum* decline markedly under a grass ley. It has been suggested that the melanised hyphae of *V. albo-atrum* are stimulated to germinate by grass roots but are unable to colonise them and subsequently die. 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 compared with a mean value of 25.9 for untreated plots planted with trees. After one year under ryegrass, the level was unchanged, at 29.7 cfu/g. After two years, the level had increased slightly to 36.4 cfu/g. After three years it had fallen to 14.3 cfu/g.

V. dahliae microsclerotia appear to be more resistant to decay than *V. albo-atrum* survival structures. No broad-leaf weeds were found in the area when soil samples were collected in January and December 2008 so it is unlikely that the *V. dahliae* persisted on roots of alternative hosts.

Table 21: Levels of *V. dahliae* in the soil – March 2005 to December 2008

Plot	Treatment	*V. dahliae* (cfu/g soil) Mar 05	Jan 06	Jan 07	Jan 08	Dec 08
1	Untreated	7.0	28.0	31.7	55.6	58.0
2	Basamid/Sistan 51	11.7	0.5	1.1	3.5	9.2
3	Untreated	9.3	7.6	16.4	38.9	59.6
4	Chlorofume	9.3	0.1	1.0	17.4	1.0
5	Chlorofume	11.3	0.6	1.7	6.5	2.5
6	Chlorofume	4.9	0.6	0.1	1.9	2.9
7	Sudan grass	9.5	7.3	18.0	36.4	36.1
8	Basamid/Sistan 51	7.5	0.4	0.1	0.8	8.4
9	BSD	10.8	7.5	1.9	37.1	30.8
10	Basamid/Sistan 51	10.7	0.0	0.0	0.3	1.3
11	Sudan grass	8.8	24.0	4.5	65.2	24.9
12	Sudan grass	10.9	30.4	7.3	38.2	50.7
13	BSD	8.0	9.9	3.0	31.2	32.8
14	BSD	14.0	4.3	3.7	18.0	20.8
15	Sudan grass	31.4	36.4	14.8	54.9	78.5
16	Basamid/Sistan 51	19.5	0.2	0.0	2.1	0.5
17	Basamid/Sistan 51	20.6	0.7	4.2	4.3	8.4
18	Sudan grass	16.8	17.1	10.6	21.0	24.2
19	Untreated	17.8	62.2	20.4	47.6	26.7
20	BSD	38.2	26.1	15.7	43.6	15.2
21	Chlorofume*	26.3	16.4	0.1	0.5	0.2
22	Untreated	13.4	33.2	24.3	34.7	82.8
23	BSD	9.7	2.9	4.0	8.4	8.7
24	Chlorofume	17.4	0.1	0.3	1.8	0.9
25	Untreated	32.6	38.1	36.8	41.3	23.4
Ryegrass strip		-	-	30.8	29.7	36.4**

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

** In December 2009 the level under ryegrass had fallen to 14.3 cfu/g.

Table 22: 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 March 05	Post-treatment levels Jan 06	Jan 07	Jan 08	Dec 08
1. Untreated	16.0	33.8	25.9	43.6	50.1
2. Sudan grass	15.5	23.0	11.1	43.1	42.9
3. BSD	16.1	10.1	5.7	27.7	21.7
4. Chlorofume	14.3	2.5*	0.6	5.6	1.5
5. Basamid/Sistan 51	13.6	0.4	1.1	2.2	5.6
Significance	0.697	<0.001	<0.001	<0.001	<0.001
LSD (15 df)	4.27	13.71	6.27	14.5	20.9

* Mean of 4 plots.

Effect of tree removal on soil level of *V. dahliae*

The effect of grubbing *Acer* and *Tilia* trees infected by Verticillium wilt on subsequent levels of *V. dahliae* in the soil is unknown. With potato crops affected by Verticillium wilt, there can be a large increase in *V. dahliae* levels in the soil in the 12 months after harvest as infested haulm rots down and releases microsclerotia into the soil. In our project, infected *Acer* and all other trees were grubbed in April 2009. The levels of *V. dahliae* 3 months pre-grubbing (December 2008), and 9 months after grubbing (December 2009) were compared. In December 2008 the mean level of *V. dahliae* was 24.3 cfu/g and this was virtually unchanged (22.8 cfu/g) one year later (Table 23). This lack of increase is probably due either to the slower decay of tree roots compared with potato haulm, and/or less production of verticillium microsclerotia on tree roots compared with potato stems.

Table 23: Effect of grubbing trees infected by Verticillium wilt on level of *V. dahliae* in the soil 9 months later

Soil area	Level of *V. dahliae* (cfu/g)	
	Pre-grubbing	Post-grubbing
A	25.8	20.9
B	21.4	19.8
C	25.7	27.6
Mean	24.3	22.8

Progression of tree death with time

Data from all plots were combined in order to examine the progression of tree death in all three species, over the three years that trees were grown on the site.

When calculating the occurrence of dead trees, trees that were completely dead or were missing were included in the category; trees with a dead top but a live base were excluded. Results on the incidence of dead trees were expressed both as a proportion of the trees remaining at the time of the assessment (i.e. excluding those previously cut down for laboratory examination), and as a proportion of all trees planted (i.e. a cumulative score). It should be noted that the calculation of cumulative values is likely to result in an underestimate of the incidence of tree death that might have occurred if all trees had been left to grow for the full three years. This is because one would expect a tree that is growing in soil infested with *V. dahliae* for three years is more likely to become infected and die than one that has been growing for one or two years in the soil

The cumulative proportion of *Acer* trees that had died by the end of the experiment (5.3%) was greater than that of *Tilia* (3.0%) or *Sorbus* (2.4%) (Table 24). There was a greater increase in death of *Acer* trees in the third year (2008), than either of the previous years. The result is not unexpected given the high incidence of suspect Verticillium wilt symptoms in 2007. *V. dahliae* was recovered from the stem base of many *Acer* trees, both with and without suspect symptoms of the disease.

Most of the *Sorbus* tree death occurred in the first year, with a slight increase in the second year and none in the third year. It is likely that death of *Sorbus* trees was primarily due to poor establishment in the dry summer of 2006. Secondary infection with coral spot (*Nectria cinnabarina*) was noticed on many of the dead *Sorbus* trees. No *V. dahliae* was recovered from any of the *Sorbus* stem bases tested in the laboratory.

The occurrence of dead *Tilia* trees increased steadily with time, from 1.4% in 2006 to 2.3% in 2007 and 3.0% in 2008. This is consistent with the increasing occurrence of suspect Verticillium wilt symptoms in *Tilia* trees, and the increasing recovery of *V. dahliae* from stem bases, with time.

Table 24: Occurrence of dead trees at yearly intervals after planting

Species	Dead[a] trees (%) in autumn		
	2006	2007	2008
As % of remaining trees[b]			
Acer	1.0	2.9	13.9
Sorbus	1.7	2.9	3.1
Tilia	1.4	2.8	5.2
As % of all trees planted[C]			
Acer	1.0	1.9	5.3
Sorbus	1.7	2.4	2.4
Tilia	1.4	2.3	3.0

[a] Dead and missing trees were classed as dead; trees with a dead top but live base were excluded.
[b] Number of trees assessed = 1080, 720 and 360 in 2006, 2007 and 2008 respectively; 360 trees of each species were cut down each autumn.
[c] Number of dead trees removed in 2006 and 2007 added to the number of dead trees observed in 2007 and 2008.

Conclusions

Year 1 (April 2005 - March 2006)

1. Chlorofume, Basamid/Sistan 51 and BSD all significantly reduced levels of *V. dahliae* microsclerotia in soil in untreated plots. The two chemical fumigants (95-97% reduction) were significantly more effective than the BSD treatment (36% reduction).

2. In autumn 2005, Chlorofume and a combined treatment of Basamid (surface layer) and Sistan 51 (injected at 25-35 cm depth) significantly reduced the number of stubby root nematodes. Incorporation of sudan grass, and a BSD treatment using rye grass, were ineffective.

3. Levels of *V. dahliae* in untreated fallow soil increased by 139% between March 2005 and January 2006. This may have been due to the decay of infected host plants releasing microsclerotia into the soil.

4. Fat hen (*Chenopodium album*) was confirmed as a host of *V. dahliae*.

Year 2 (April 2006 - March 2007)

1. At 15 months after soil treatment, soil levels of *V. dahliae* were significantly reduced by sudan grass as well as by BSD, Chlorofume and Basamid / Sistan 51.

2. The mean level of *V. dahliae* in untreated soil declined from 33.8 to 25.9 cfu/g between January 2006 and January 2007.

3. Pre-plant soil treatment with Basamid/Sistan 51 significantly reduced infection of *Acer* by *V. dahliae*. Chlorofume appeared to reduce infection. Sudan grass and BSD did not reduce infection.

4. The mean incidence of *Acer* trees infected in the stem base by *V. dahliae* at six months after planting ranged from 3% (after Basamid/Sistan 51) to 43% (after BSD), compared with 33% in untreated plots. The greatest level of infection recorded in an individual plot was 12 out of 15 trees. No infection was detected in *Tilia* or *Sorbus* trees at this time

5. These initial results after 6 months growth indicated a trend for the incidence of verticillium infection in *Acer* to increase above a threshold of 0.5 cfu/g of *V. dahliae* in the soil.

6. Isolation of *V. dahliae* from *Acer* stem bases was significantly associated with the occurrence of internal staining of the wood, although not exclusively so.

7. *Acer platanoides* ‘Emerald Queen’ is more susceptible to *V. dahliae* than *Tilia cordata* ‘Greenspire’ and *Sorbus aria* ‘Majestica’ on *S. intermedia* rootstock.

8. Basamid/Sistan 51 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.

<u>Year 3 (April 2007 - March 2008)</u>

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 possibly indicate release of *V. dahliae* microsclerotia into the soil from decaying roots of infected *Acer* or *Tilia* trees.

2. After one year under a ryegrass sward, there was no decrease in the level of *V. dahliae* in the soil. Presumptive Verticillium wilt symptoms first occurred in *Acer* and *Tilia* in the second growing season after planting in land infested with *V. dahliae*. No symptoms suggestive of Verticillium wilt occurred in *Sorbus*.

3. The overall proportion of *Acer* trees found infected in the stem base in winter 2007 (32%) was similar to that found infected in 2006 (36%).

4. Pre-plant soil treatment with Basamid/Sistan 51 and Chlorofume significantly reduced infection of *Acer* and *Tilia* stem bases by *V. dahliae*. BSD reduced infection in *Tilia* but not in *Acer*. Sudan grass did not reduce infection in either species.

5. After two growing seasons, *Acer platanoides* was clearly 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 plating method, resulted in 10–12% infection in *Acer* and nil in *Tilia* after 18 months’ growth.

6. Basamid/Sistan 51 and Chlorofume significantly reduced low levels of stubby root and stunt/spiral nematodes; sudan grass and BSD were ineffective.

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

<u>Year 4 (April 2008 - March 2009)</u>

1. Mean soil levels of *V. dahliae* in December 2008 had changed little from one year earlier.

2. After two years under a ryegrass sward, there was little change in the level of *V. dahliae* in soil.

3. Bark cracking incidence and severity in *Acer* was significantly reduced in autumn 2008 by Chlorofume and Basamid/Sistan 51. Leaf yellowing in *Tilia* was not affected by pre-plant soil treatment.

4. After growth for three summers, a low level of infection (3%) also occurred in *Tilia cordata* planted on ground with <0.7 cfu/g as measured by the ADAS soil test. The incidence of infection in *Tilia* was much greater at soil levels < 10 cfu/g (24%). In comparison, *Acer* showed infection of over 40% at levels <10 cfu/g even after two summers. This result suggests that soil levels of *V. dahliae* can be used as a broad strategy to determine the risk of planting *Acer platanoides* and *Tilia cordata*.

5. A regression of *V. dahliae* soil infestation level in January 2006, just before planting, against the total proportion of *Tilia* trees infected (2007 + 2008) was significant (P<0.001) and accounted for 57% of the variance.

6. The overall proportion of *Tilia* trees found infected in 2008 (16%) was slightly greater than that found infected in 2007 (10%). None was found to be infected in 2006.

7. There was no difference in the recovery of *V. dahliae* from stained and unstained transverse sections of wood taken from the base of *Tilia* trees.

8. Pre-plant soil treatment with either BSD, sudan grass, Basamid/Sistan 51 or Chlorofume, significantly increased mean circumference of *Acer* and *Tilia* by around 2 – 3 cm after three seasons. The circumference of the *Sorbus* trees was increased by the two chemical treatments only.

<u>Year 5 (April 2009 - March 2010)</u>

1. After three years under a ryegrass sward, the level of *V. dahliae* in soil had fallen from 30.8 cfu/g to 14.3 cfu/g.

2. The effect of grubbing trees infected by Verticillium wilt on subsequent levels of *V. dahliae* in the soil was investigated The mean level of *V. dahliae* shortly before grubbing was 24.3 cfu/g, and 9 months later (December 2009) was virtually unchanged at 22.8 cfu/g.

Technology transfer

<u>Meetings</u>

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

Project steering group meeting, East Malling, 13 March 2007.

Project sheeting group meeting, East Malling, 21 February 2006.

Project steering group meeting, Andlers Ash, Hants, 12 May 2005.

<u>Articles</u>

O'Neill TM (2010). Looking for a clean start. *HDC News* **155,** 22–23.

O'Neill TM (2008). Restraints on wilt. *HDC News* **144**, 28-31.

O'Neill TM (2006). Cleaner land for healthier trees. *HDC News* **129**, 16-17

<u>Presentations</u>

Progress in soil disinfestation – chemical, physical and biological. HTA Tree and Hedging Conference, Wellesbourne, 22 September 2005 (Tim O'Neill).

Soil disinfestation options for control of Verticillium wilt in trees. IPPS Conference, Ipswich, 8 October 2010 (Tim O'Neill) (planned).

<u>Edited paper</u>

O'Neill TM, Locke T & Dyer C (2010). A comparison of four pre-plant soil treatments for control of Verticillium wilt in field-grown trees. *Journal of Agricultural Science and Technology* (in press).

Poster

O'Neill TM, Locke T & Dyer C (2009). A comparison of four pre-plant soil treatments for control of Verticillium wilt in field-grown trees. Seventh International Symposium on Chemical and non-chemical Soil and Substrate Disinfestation, 13-18 September 2009, Leuven, Belgium.

Acknowledgements

We are grateful to Phillip Hall of Hillier Nurseries for managing the site, to Chris Dyer of ADAS for statistical advice, and to Angela Huckle and Dan Smith for field and laboratory work, and to Hossein Arshadi (Hillier Nurseries Ltd) and John Adlam for helpful discussion.

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.

A more comprehensive list of references is given in the 2006, 2007 and 2008 Annual Reports.

Appendix 1: Individual plot data – level of *V. dahliae* in soil and numbers of *Acer* and *Tilia* trees from which the fungus was recovered.

Treatment	Plot N°	Level of *V. dahliae* (cfu/g)		No. *Acer* trees (of 15) infected		No. *Tilia* trees (of 15) infected	
		Mar 05	Jan 06	Oct 06	Oct 07	Oct 07	Dec 08**
1. Untreated	1	7.0	28.0	0	9	1 (12)	3
	3	9.3	7.6	4	7 (13)	2	1
	22	13.4	33.2	5	6	2 (14)	3
	19	17.8	62.2	5	3	5 (14)	4
	25	32.6	38.1	11	0	3	5
2. Sudan grass	11	8.8	24.0	5	11	5	3
	7	9.5	7.3	7	5 (14)	0	1
	12	10.9	30.4	7	5	1	2
	18	16.8	17.1	4	4	1	0
	15	31.4	36.4	3	7	8 (14)	3
3. BSD	13	8.0	9.9	12	10	4	1
	23	9.7	2.9	2	4	0	3
	9	10.8	7.5	4	7	1	0
	14	14.0	4.3	7	8	0	0
	20	38.2	26.1	7	6	2	2
4. Chlorofume	6	4.9	0.6	7	4	0	1
	4	9.3	0.1	2	2	0 (14)	0
	5	11.3	0.6	0	3	0	0
	24	17.4	0.1	1	1 (14)	0	0
	21*	26.3	16.4	*	*	*	*
5. Basamid / Sistan 51	8	7.5	0.4	0	2	0	0
	10	10.7	0	0	3	0	1
	2	11.7	0.5	0	4 (14)	0	2
	16	19.5	0.2	0	1	0	2
	17	21.6	0.7	2	2	0	1

* Not planted with trees

** Only 10 trees were assessed in Dec 08

( ) – number of trees assessed where less than 15

Appendix 2: Crop diary: January 2008 - December 2009

7 August 2008	– Leaf yellowing assessed
19 November 2008	– *Acer* bark splitting assessed
19–20 November 2008	– Growth measured
11 December 2008	– Soil sampled to determine *V. dahliae* levels
12 December 2008	– *Tilia* trees sampled (remaining third removed)
April 2009	– All remaining trees grubbed out and soil sub-soiled, ploughed and drilled with Italian ryegrass.
December 2009	– Soil sampled to determine *V. dahliae* levels