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

HNS 137

March 2007

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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

1. Headline

Thirty-three percent of *Acer* trees planted on soil infested with *Verticillium dahliae* became infected within 6 months of planting, although none developed symptoms of the disease during this period; pre-plant soil treatment with Basamid/Sistan 51 reduced the incidence of infection to 3%.

2. Background and expected deliverables

The value of the 1535 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. Up 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 application of methyl bromide for pre-plant soil use was prohibited from 1 January 2005, apart from Critical Use Exemptions (CUE). A CUE for field grown trees and hardy nursery stock of 2.5 tonnes was granted for 2006, following a request for 12 tonnes. From 1 January 2007, soil disinfestation in 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 three 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 three 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).

3. Summary of the project and main conclusions

Detail of soil disinfestation treatments

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/m²), 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/m² (maximum permitted rate) by contractor on 26 August.
5. Basamid (98% dazomet) applied at 45 g/m² and incorporated to 20 cm depth, and Sistan 51 (51% metam sodium) injected at 25-35 cm depth at 90 mL/m² (= 46 mL/m² 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. .

Efficacy of soil treatments

Reduction of *V. dahliae* levels in soil:

In January 2007, the mean level of *V. dahliae* in untreated soil (25.9 cfu/g) had declined 23% over that found one year earlier (33.8 cfu/g) (Table 1). The mean level of *V. dahliae* in all of the treated soils was significantly lower that in untreated soil. The two chemical treatments were particularly effective. The effectiveness of Sudan grass appeared to have increased between January 2006 and January 2007, possibly indicating that this treatment affects *V. dahlia* viability over a period greater than three months (the period between incorporation of Sudan grass and the first post-treatment measurement of *V. dahliae* soil levels).

Levels of *V. dahliae* in untreated, fallow soil increased by 139% between March 2005 and January 2006, and then declined by 23% over the next year.

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
Untreated	16.0	33.8	25.9
Sudan grass	15.5	23.0 (40)	11.1 (57)
BSD	16.1	10.1 (70)	5.7 (78)
Chlorofume	14.3	2.5 (93)	0.6 (98)
Basamid/Sistan 51	13.6	0.4 (79)	1.1 (96)

( ) – percentage reduction compared with untreated soil

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. One third of the trees in each plot was cut down after 6 months and the stem bases were tested for infection by *V. dahliae.* None of the trees showed definitive symptoms of verticillium wilt at this time. However, *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%) and Chlorofume (17%) (Fig.1). Neither Sudan grass nor BSD reduced the incidence of infection. There was a strong association between staining of *Acer* stem base wood and recovery of *V. dahliae*, although the fungus was recovered from some unstained stem bases and *vice-versa*. No *V. dahliae* was recovered from *Tilia* or *Sorbus* stem bases.

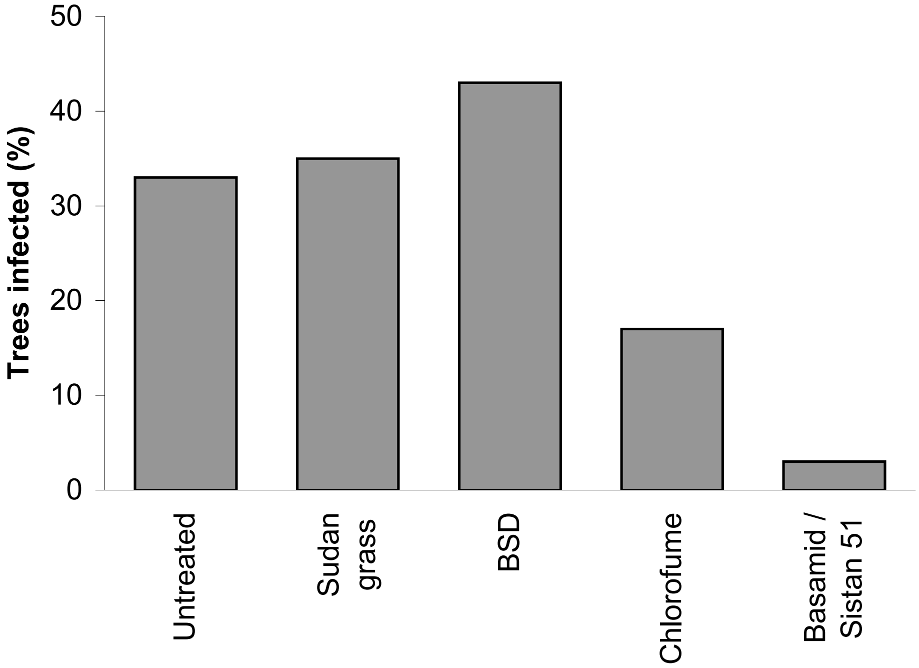

Figure 1: Effect of pre-plant soil treatment on infection of *Acer* by *V. dahliae* at 6 months after planting

Soil level of V. dahliae *and risk of infection in* Acer

There was evidence that the incidence of *Acer* trees infected by *V. dahliae* at 6 months after planting was related to the level of *V. dahliae* in the soil at planting, with an increase at soil levels above 0.5 cfu/g. The Basamid/Sistan 51 treatment reduced soil levels of *V. dahliae* to an average of 0.4 cfu/g , and only 4% of Acer trees planted on this land became infected. The Chlorofume treatment reduced *V. dahliae* to 2.5 cfu/g and 17% of trees became infected. Using information on the incidence of infection from across all treatments, the mean percentages of infected *Acer* trees, after planting in soil with *V. dahliae* levels of 0.1-0.5 cfu/g, >0.5-10.0 cfu/g and >10.0 cfu/g, were 4%, 31% and 35% of trees respectively.

It should be noted that none of the infected trees showed definitive symptoms of verticillium wilt at the time of sampling, just 6 months after planting. It is possible that these trees might not have developed symptoms of the disease in subsequent years, if, for example, infection was restricted to a small proportion off xylem in the tree circumference. It is anticipated that when trees are assessed in autumn 2007, 18 months after planting, there is a greater chance that some trees will be showing symptoms of the disease and it may then be possible to determine the effect of treatment on disease development, as well as on stem base infection.

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

Work on this task was delayed due to failure to obtain significant and consistent recovery of *V. dahliae* DNA from soil.

Crop and weed hosts of V. dahliae

V. dahliae has been recorded on an extremely wide range of herbaceous and woody dicotyledonous plants. Although monocotyledonous plants (eg wheat, barley and grasses) have been recorded as symptomless carriers of the fungus (Smith *et al.*, 1988), there is evidence that root exudates from grasses and cereals cause microsclerotia touching the growing roots to germinate, and that the fungus colonises the roots but does not infect the plant; consequently the fungus fails to produce more microsclerotia to colonise the soil. By causing the microsclerotia to germinate, the grass and cereal roots directly lead to a decline in microsclerotia numbers in the soil. This can lead to a very significant decline in soil infestation by *V. dahliae* over a period of 4-7 years if the field is cropped with Graminaceous plants, provided dicotyledonous weed hosts are not allowed to grow in the grass or cereal crops.

Among the herbaceous hosts are various species of Cucurbitaceae, Compositae, Malvaceae, Rosaceae and Solanaceae. Woody hosts include ornamental trees, especially species of *Acer*, *Catalpa*, and *Koelreuteria*, also *Prunus* and seedling *Quercus*. It should be noted that the listing of a species as a host of *V. dahliae* does not indicate how commonly the species is infected by *V. dahliae* in nature. Some hosts of *V. dahliae* recorded in the UK (Anon., 2007; Baker, 1972; Moore, 1959) are listed in Table 2. Crops that are known to be very susceptible include linseed, potato and strawberry.

In the first year of this project we recorded *V. dahliae* at the experimental site in roots and the stem base of fat hen (*Chenopodium album*). *Tagetes* is recorded as a host of the fungus and circumstantial evidence suggests that a crop of *Tagetes* grown in the field prior to establishment of the trial was infected. The increase in *V. dahliae* in the first year is thought to be due to their release from the decaying *Tagetes* plant material.

Many weed species have been recorded as hosts of *V. dahliae* in other countries. Recently a survey of weed species in Greece reported the most susceptible weed species (isolation frequency 28-53%; moderate disease severity) to be shepherd`s purse (*Capsella bursa-pastoris),* hoary pepperwort (*Cardaria draba), Chenopodium album,* groundsel (*Senecio vulgaris)* and black nightshade *(Solanum nigrum).* Less susceptible species (isolation frequency 5-15%; slight disease severity) were *Amaranthus* sp., *Amaranthus melanolepis*, *Convolvulus arvensis*, *Erodium* sp., *Euphorbia* sp., sun spurge (*Euphorbia helioscopia)*, *Helminthotheca echioides*, prickly lettuce (*Lactuca serriola)*, common mallow (*Malva sylvestris)*, white charlock (*Raphanus raphanistrum)*, white mustard (*Sinapis alba)*, charlock (*Sinapis arvensis)*, sow thistle (*Sonchus oleraceus)* and *Trifolium* sp. (Ligoxigakis *et al*., 2002). Many of these weeds are commonly found in the UK, but the significance of any infection in these hosts in our cooler climate and soil temperatures is unknown.

Table 2: Some hosts of *Verticillium dahliae* recorded in the UK

Host genus*	English name**
Acer	Maple, sycamore
Alstroemeria	
Anchusia	
Anthemis	
Apium	Celery
Aster	Michaelmas daisy
Castanea	Sweet chestnut
Cercis	Judas tree
Chrysanthemum	
Cotinus	Smoke tree
Crataegus	Hawthorn
Cucumis	Cucumber, melon
Cydonia	Quince
Dahlia	
Daphne	
Delphinium	
Exochorda	
Fragaria	Strawberry (fairly common)
Geranium	
Helenium	
Helichrysum	
Humulus	Hop
Helianthus	Sunflower
Lactuca	Lettuce
Lathyrus	Sweet pea
Ligustrum	Privet
Linum	Linseed (common)
Lupinus	Lupin
Lycopersicon	Tomato
Lythrum	
Malus	Apple (rare)
Medicago	Sainfoin
Mentha	Mint (quite common)
Onobrychis	Lucerne
Papaver	Poppy
Phaseolus	Runner bean
Philadelphus	Mock orange
Phlox	
Pisum	Pea
Prunus (rare)	Cherry, peach, plum
Pyrus	Pear

Ribes	Currant, gooseberry
Rhus cotinus	Sumach
Rosa	Rose
Rubus	Blackberry, raspberry
Salvia	Sage
Scabious	
Solanum	Potato (common)
Tilia	Lime
Trifolium	Clover
Viburnum	
Vitis	Vine

*Some genera include both susceptible and resistant species.
**A note on the frequency that *V. dahliae* has been found associated with particular hosts, in the UK, is given where known.

4. 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.

5. Action points for growers

- Do not plant *Acer platanoides* on land infested with *V. dahliae*. After just 6 months, 36% of trees planted on land infested with 1-68 cfu/g had become infected by *V. dahliae*; even on land with 0.1-1.0 cfu/g, 10% of trees had become infected.

- Soil treatment with Basamid/Sistan 51 and Chlorofume, at the rates described in this report, can reduce soil levels of *V. dahliae* by over 90%. 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).

- Many dicotyledonous weeds that are common in the UK, 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* (eg 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.

SCIENCE SECTION

Introduction

In the first stage of this experiment, two chemical soil disinfestation treatments (Basamid/Sistan 51 and Chlorofume) and 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. Trees will be examined for 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, will be examined.

Materials and methods

Pre-plant soil disinfestation treatments

Soil treatments applied in autumn 2005 are detailed below.

Table 1: Summary of pre-plant soil treatments

Treatment no.	Trade Name	Active ingredient	Application Rate	Application Regime
1	Untreated control	-	-	-
2	Sudan grass	Sudan grass grown on plots (3.5 kg/ha drilled)	3 kg/m^2 fresh weight incorporated 6 Sep	Cut, ploughed, power harrowed and rolled
3	Biological Soil Disinfestation (BSD)	Rye-grass grown on plots (50 kg/ha drilled)	5.7 kg/m^2 fresh weight incorporated 9 Aug	Flooded with 75 mm water, spaded and covered with VIF
4	Chlorofume	99.5% chloropicrin	Injected to 35 cm depth at 40 mL/m^2, 26 Aug	Single application, covered with plastic sheets
5	Basamid &	98% dazomet	45 g/m^2 incorporated to 20 cm depth	Sistan injected then Basamid applied, rolled and covered with plastic sheet
	Sistan 51	51% metam sodium	90 mL/m^2 injected and incorporated to 25-35 cm depth, 26 Aug	

Experiment design

Treatments were arranged in a randomised block, split-split-plot design with five replicates of five treatments (i.e. 25 main plots). Main plots were soil treatments, sub-plots were replicates (15 within each main plot) and sub-sub-plots were tree species (three species).

Tree planting

The treated soils were planted with two species reported to be susceptible to *V. dahliae* and one species (*Sorbus aria*) that is susceptible to replant disease:

Acer platanoides rootstock budded with `Emerald Queen′
Tilia cordata rootstock budded with `Greenspire′
Sorbus intermedia rootstock budded with *Sorbus aria* `Majestica'

The trees were sourced from a supplier with no history of verticillium wilt. A random sample of 50 trees of each species was examined in March 2006 to check the health of the planting stock. Isolations were made from three transverse sections of each stem base. Samples were plated onto agar, incubated and examined for fungal growth characteristic of *V. dahliae*.

Each main plot was planted with 45 trees of each species on 26 April 2006 (135 trees in total), and surrounded by a single guard row of *Betula pendula*. Main plot 21 was left unplanted because of the failure to treat this plot with Chlorofume as planned. The different species were arranged in a randomised order in 15 replicates; each replicate contained a group of three plants of each species. Trees were planted 40 cm apart along a row with 1.8 m between rows. Following destructive assessment of one third of the trees in October 2006, and a further third to be removed in October 2007, the remaining trees will be 1.2 m apart along a row.

Occurrence of Verticillium wilt symptoms – October 2006

Field assessment:
Trees were assessed on 11 October to determine the occurrence of stem splitting (bark cracking) suggestive of verticillium wilt, leaf yellowing and the incidence of dead 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 for laboratory testing for *V. dahliae.* A sample of yellowing leaves was collected from three *Acer* trees and the petioles were tested for *V. dahliae.*

Laboratory tests:

Stem bases were stored at 4ºC until required. Each stem was examined for external and internal wood staining. The staining level categories were defined as follows:

External (under bark staining):

0 – None	No visible staining.
1 – Light	Pale brown/green staining in streaks, only partially covering circumference of stem.
2 – Medium	Dark brown/green staining in streaks, partially or completely covering the circumference of stem.
3 – Heavy	Dark brown/green staining with some very dark/black streaks, often covering whole circumference of stem.

Internal (wood staining):

0 – None	No visible staining.
1 – Light	Pale brown/green staining, often with a blotchy appearance.
2 – Medium	Dark brown/green staining, often in rings, rays or irregular patches, sometimes stain covered whole stem section.
3 – Heavy	Dark brown/green staining with some very dark/black spots, often in bands, semicircular/irregular patches, and sometimes covering whole stem section.

For all *Acer* stems, a 10 cm length from the basal end was surface-disinfected in sodium hypochlorite (1% available chlorine, 1 minute), and the was bark removed. Three sections (4-6 mm thick) were sawn off from the basal 10 cm, disinfected in alcohol (30 s), placed onto PDA+streptomycin and incubated at 22ºC in the dark. After 4 days, the vascular tissue was examined for the white, fluffy mycelium of *V. dahliae.* Agar plates were re-examined after 14-21 days for mycelial growth and microsclerotia of *V. dahliae* that had developed from the wood onto the agar. For *Sorbus* and *Tilia*, samples of approximately five stems from each internal staining level (where available) and five unstained stems were examined for *V. dahliae* by plating onto agar. The samples were taken from plots with a range of soil levels of *V. dahliae.*

<u>Levels of *V. dahliae* in the soil – January 2007</u>

Soil samples were taken from each plot on 12 January 2007 and tested for *V. dahliae*. Fifty cores were taken to a depth of 20 cm, taking 12 cores from 2 rows and 13 cores from the other two rows. Samples were taken at regular intervals along the row and at c. 30 cm from the base of the nearest tree. Soil samples were sent to ADAS High Mowthorpe, for laboratory analyses of *V. dahliae* in the soil using the standard agar-plating method.

Determination of *V. dahliae* in soil by a PCR test

As part of HDC project SF 70, work is in progress to develop a real-time polymerase chain reaction (PCR) test to determine levels of *V. dahliae* DNA in soil. 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.

Results and Discussion

Pre-plant examination of trees for *V. dahliae*

Isolation from a random sample of 50 stem bases in March 2006, immediately before planting, confirmed the presence of *V. dahliae* in one *Acer* stem (2% of trees); no *V. dahliae* was detected in the stem base of similar samples of the *Sorbus* or *Tilia* trees.

Field assessment – October 2006

Longitudinal bark splitting on the main stem suggestive of *V. dahliae* infection was found in around 21% of *Acer* trees and not at all in *Tilia* or *Sorbus*. Leaf yellowing occurred in around 5% of *Acer* and occasional *Tilia* trees. There was no obvious difference between treatments in the incidence of *Acer* trees with stem splits or leaf yellowing, or the total number of dead trees, when examined by pre-plant soil treatment (Table 3.1). Nor did these symptoms appear to be associated with levels of *V. dahliae* in the soil (Table 3.2)

Table 3.1: Occurrence of stem splits, leaf yellowing and dead trees according to pre-plant soil treatment –field assessment, October 2006

Treatment	Mean No. *Acer* trees (of 45 assessed)*		Total number trees dead
	Split stem	Yellow leaves	(of 135)
1. Untreated	10.8	3.2	9
2. Sudan grass	7.2	1.2	5
3. BSD	12.6	3.2	13
4. Chlorofume	6.3	0.3	13
5. Basamid/Sistan	9.8	4.6	8
Mean (%)	9.3 (21%)	2.5 (5%)	9.6 (7.1%)

* 5 replicates for all treatments except Chlorofume (4 replicates)

Table 3.2: Occurrence of stem splits, leaf yellowing and dead trees according to soil levels of *V. dahliae* prior to planting –field assessment, October 2006

Level of *V. dahliae* (propagules/g) in January 2006	Number of plots	Mean No. *Acer* trees (of 45) with		Total number trees dead (all species)
		Split stem	Yellow leaves	
0 - 5	9	3.3	9.0	2.1
5.1 - 20	7	0	8.6	2.0
20.1 - 70	8	3.3	10.6	1.9

Laboratory assessments

No *V. dahliae* was recovered from petioles of the sample of yellowed leaves collected in October 2006.

Staining colours and patterns in stem base wood of the three different tree species was as follows:

Acer: Wood was stained black, dark green and reddish brown (often all present at the same time). Internal staining patterns generally included dark spots, rings, rays and semicircular to irregular patches (occasionally a whole stem section was discoloured).
Sorbus: Wood was stained reddish to dark brown (with occasional black spots). Internal staining patterns generally comprised discolouration over the whole stem section (with occasional darker brown/black patches).
Tilia: Wood was stained black (often with a purple tinge), reddish to dark brown, and grey (often all present at the same time). Internal staining patterns generally comprised of dark spots, often in ring patterns, with a grey discolouration over the whole stem section.

In *Acer*, there was a high incidence of both external and internal wood staining (Table 3.3). The incidence and severity of external staining were significantly reduced by the Basamid/Sistan 51 treatment. Internal staining was generally observed wherever external staining was visible and *vice-versa*.

In *Sorbus* and *Tilia*, there was only a low level of wood staining, which differed in appearance from that found in *Acer*. Pre-plant soil treatment had no obvious effect on the incidence of stem base staining in the *Sorbus* and *Tilia* trees (Table 3.4).

Table 3.3: Effect of pre-plant soil treatment on the occurrence of internal and external wood staining in *Acer* – October 2006

Treatment	External staining		Internal staining	
	Mean No. stems (of 15)	Mean severity (0-3)	Mean No. stems (of 15)	Mean severity (0-3)
1. Untreated	11.2	1.5	11.6	1.5
2. Sudan grass	10.2	1.5	11.0	1.5
3. BSD	13.4	1.9	12.2	1.9
4. Chlorofume	12.3	1.5	12.8	1.4
5. Basamid/Sistan	6.6	0.7	7.2	0.7
Significance	0.022	0.022	0.145	0.065
LSD	4.02	0.68	4.67	0.75

Table 3.4: Effect of pre-plant soil treatment on occurrence of internal wood staining in the stem bases of *Sorbus* and *Tilia* – October 2006

Treatment	Mean number of trees (of 15) with staining	
	Sorbus	*Tilia*
1. Untreated	0	1
2. Sudan grass	1	0
3. BSD	2	0
4. Chlorofume	0	0
5. Basamid/Sistan	1	0

Data not analysed due to low occurrence of staining

V. dahliae was isolated from the stem bases of 95 out of a total of 359 *Acer* trees tested (26.4%). The mean number of trees (out of 15) from which *V. dahliae* was isolated was significantly reduced from 5.0 in untreated plots to 0.4 by Basamid/Sistan 51 treatment (Table 3.5). Chlorofume appeared to reduce the incidence of infected trees although this difference was not quite significant at the 5% level. Neither Sudan grass nor BSD significantly reduced the incidence of trees infected by *V. dahliae*.

Given the low level of *V. dahliae* recorded in the *Acer* trees pre-planting (2%), and the large differences in the incidence of infection associated with different soil treatments (ranging from 3% to 43%), it seems probable that the majority of infection in trees arose from *V. dahliae* in the soil.

Recovery of *V. dahliae* from *Acer* stem bases was significantly ($p<0.001$) associated with occurrence of staining within the stem-base wood (Table 3.6), although the fungus was also recovered from some non-stained wood and was not recovered from some stained wood. This probably explains why there was no significant difference between treatments in the incidence of trees with internal wood staining (Table 3.3), and suggests that assessment of wood staining as an indication of *Verticillium* infection in trees would be less sensitive than isolation of the fungus from tree bases. Recovery from some unstained wood is expected as the fungus could be present in advance of any tissue staining, which develops in response to the presence of the fungus. Equally, the failure to recover *V. dahliae* from some stained wood may be because the chemical alterations that lead to wood staining had killed the fungus.

When *V. dahliae* was recovered from one of the three sections taken from each *Acer* stem base, it was generally also recovered from one or both of the other two sections (Table 3.7).

Recovery of *V. dahliae* from *Acer* stem bases was not associated (p=0.073) with the occurrence of stem splits assessed in October 2006 (Table 3.8).

No *V. dahliae* was recovered from the sample of *Sorbus* (19) or *Tilia* (18) stem bases tested. There was no obvious association between soil level of *V. dahliae* and occurrence or level of staining in *Sorbus* and *Tilia* stems (Appendix 3).

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

The mean number of *Acer* trees per plot infected by *V. dahliae* appeared to increase as the soil infestation level of *V. dahliae* increased (Table 3.9).

Table 3.5: Effect of pre-plant soil treatment on internal wood staining and recovery of *V. dahliae* from *Acer* stem bases – October 2006

Treatment	Mean number of trees (of 15) with *V. dahliae* isolated	Mean % trees infected by *V. dahliae*
1. Untreated	5.0	33
2. Sudan Grass	5.2	35
3. BSD	6.4	43
4. Chlorofume	2.5	17
5. Basamid/Sistan	0.4	3
Significance	0.030	0.030
LSD	3.89	25.94

Table 3.6: Association of internal wood staining and recovery of *V. dahliae* from *Acer*

	Number of trees in each category*	
	Wood stained	Not stained
V. dahliae isolated	71	24
Not isolated	14	250

*359 trees examined. Pearson chi square value =186.4; p<0.001.

Table 3.7: Consistency of recovery of *V. dahliae* from *Acer* stem bases – 2006

	V. dahliae recovered from:		
Nil	1/3 sections	2/3 sections	3/3 sections
264	9	13	73

*359 trees examined

Table 3.8: Association of stem splitting in *Acer* with recovery of *V. dahliae* from the stem base of affected trees

	Number of trees in each category*	
	Stem split	Stem not split
V. dahliae isolated	9	85
V. dahliae not isolated	12	253

*359 trees examined. Pearson chi square value =3.21; p=0.073.

Table 3.9: Effect of soil infestation level with *V. dahliae* on occurrence of infection in *Acer* trees – October 2006

V. d. level in soil – Jan 06	No. plots in category	No. plots with infected trees	Mean number of trees infected/plot (of 15)	Total No trees infected
0	1	0	0	0
0.1 – 0.5	5	3	0.6	3
>0.5 – 10.0	9	6	4.7	42
>10.0	9	8	5.2	47

Levels of *V. dahliae* in soil - January 2007

The levels of *V. dahliae* recorded in individual plots in January 2007 were broadly similar to those recorded a year earlier (Table 3.10), with the mean values of untreated soil and the two biological treatments slightly lower (Appendix 4). The exception was plot 21, which was left untreated in error in autumn 2005 and was treated with Chlorofume in May 2006.

Levels of *V. dahliae* in January 2007 differed significantly between treatments (Table 3.11). Mean levels were significantly lower following all soil treatments than in untreated soil. The two chemical treatments were particularly effective. Interestingly, the soil level of *V. dahliae* after Sudan grass, which did not differ from the untreated control in January 2006, was significantly lower than the control in January 2007. Possibly this indicates that after incorporation into the soil Sudan grass affects viability of *V. dahliae* for a period greater than three months (the period between incorporation and initial measurement of *V. dahliae* soil levels).

In January 2007, the level of *V. dahliae* in soil under an area of ryegrass adjacent to the experimental area was 30.8 propagules/g. This compares with a mean value of 25.9 for untreated plots. The change of *V. dahliae* levels with time under ryegrass will be determined. It is planned that the sward will be kept free of broad-leaf weeds.

Table 3.10: Levels of *V. dahliae* in the soil – March 2005, January 2006 and January 2007

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

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

Table 3.11: 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
1. Untreated	16.0	33.8	25.9
2. Sudan grass	15.5	23.0	11.1
3. BSD	16.1	10.1	5.7
4. Chlorofume	14.3	2.5*	0.6
5. Basamid/Sistan 51	13.6	0.4	1.1
Significance	0.697	<0.001	<0.001
LSD	4.27	13.71	6.27

* Mean of 4 plots.

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

Work on this task was delayed due to failure to obtain significant and consistent recovery of *V. dahliae* DNA from soil.

Conclusions

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

2. The efficacy of Sudan grass in reducing soil levels of *V. dahliae* was greater at 15 months than at 3 months after incorporation of the grass.

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

4. Pre-plant soil treatment with Basamid (45g/m^2) plus Sistan 51 (90 mL/m^2) significantly reduced infection of *Acer* by *V. dahliae.* Treatment with Chlorofume at 40 mL/m^2 appeared to reduce infection. Treatment with Sudan grass and BSD did not reduce the incidence of infection in *Acer*.

5. The mean incidence of *Acer* trees infected by *V. dahliae* six months after planting ranged from three percent (after Basamid/Sistan) to 43% (after BSD), with 33% in untreated plots. The greatest level of infection recorded in an individual plot was 12 out of 15 trees.

6. Initial results indicate 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.

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

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

Technology transfer

Article

O'Neill, T.M. (2006). Cleaner land for healthier trees. *HDC News* **129,**16-17

Meetings

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

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Appendix 1: Individual plot data

Treatment	Plot N°	Level of *V. dahliae*		N°. *Acer* trees (of 15) infected by *V. dahliae* (Oct. 06)
		Mar 05	Jan 06	
1. Untreated	1	7.0	28.0	0
	3	9.3	7.6	1
	22	13.4	33.2	5
	19	17.8	62.2	5
	25	32.6	38.1	11
2. Sudan grass	11	8.8	24.0	5
	7	9.5	7.3	7
	12	10.9	30.4	7
	18	16.8	17.1	4
	15	31.4	36.4	3
3. BSD	13	8.0	9.9	12
	23	9.7	2.9	2
	9	10.8	7.5	4
	14	14.0	4.3	7
	20	38.2	26.1	7
4. Chlorofume	6	4.9	0.6	7
	4	9.3	0.1	2
	5	11.3	0.6	0
	24	17.4	0.1	1
	21*	26.3	16.4	*
5. Basamid/Sistan 51	8	7.5	0.4	0
	10	10.7	0	0
	2	11.7	0.5	0
	16	19.5	0.2	0
	17	21.6	0.7	2

* Not planted with trees

Appendix 2: Efficacy of soil treatments on level of *V. dahliae* at different levels of soil infestation

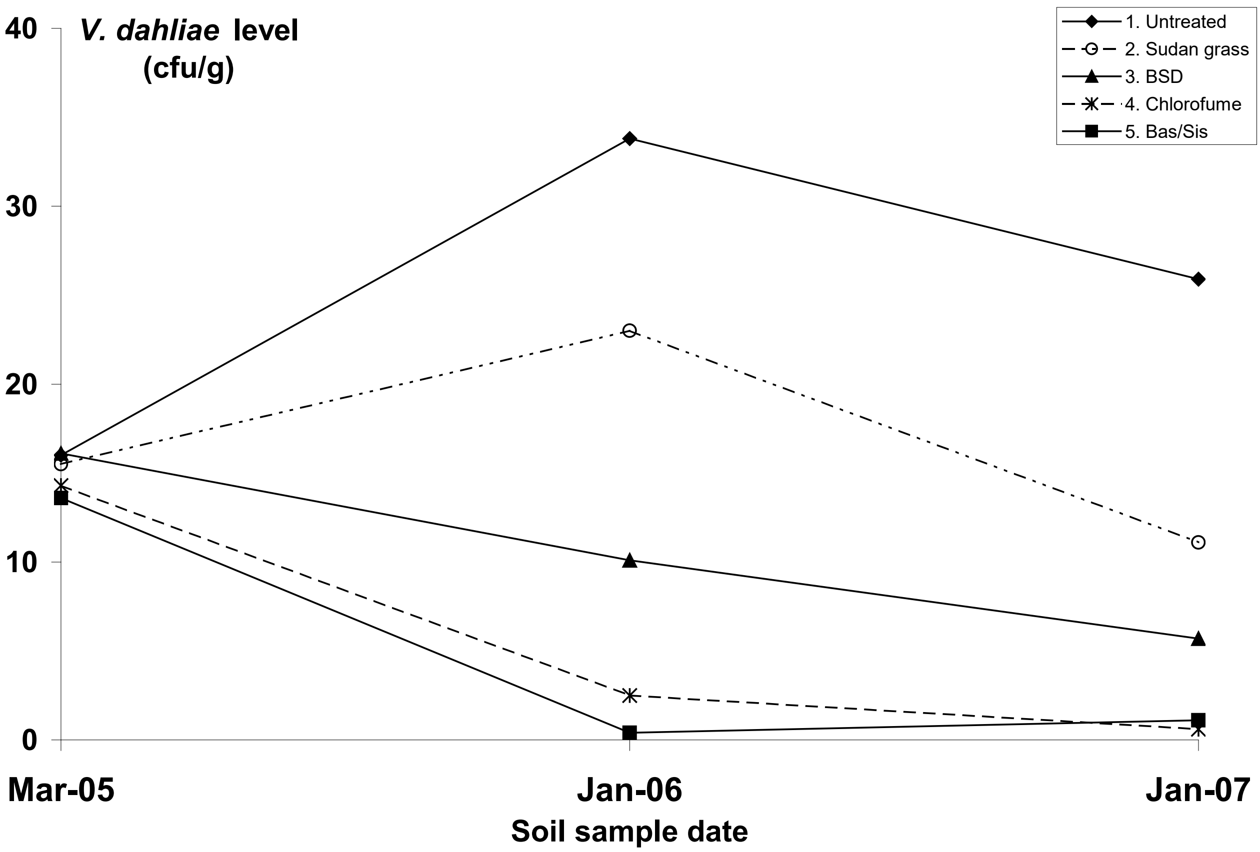
Treatment	Soil infestation (Initial level of *V. dahlia* (cfu/g), Mar. 05)	% reduction (increase) (Dec. 05–Jan. 07)
Untreated	7.0	(400)
	9.3	18
	13.4	(247)
	17.8	(349)
	32.6	(17)
Sudan grass	8.8	(273)
	9.5	23
	10.9	(279)
	16.8	(2)
	31.4	(16)
BSD	8.0	(24)
	9.7	70
	10.8	31
	14.0	69
	38.2	32
Chlorofume	4.9	88
	9.3	99
	11.3	95
	17.4	99
	26.3	*
Basamid/Sistan 51	7.5	95
	10.7	100
	11.7	96
	19.5	99
	20.6	97

* Treatment not applied until spring 06; plot not planted with trees

Appendix 3. Soil *V. dahliae* levels and staining in *Sorbus* and *Tilia* - 2006

Sorbus staining level	Associated *V. dahliae* levels in soil	*Tilia* staining level	Associated *V. dahliae* levels in soil
0	0.4	0	30.4
0	7.5	0	9.9
0	9.9	0	36.4
0	17.1	0	62.2
0	38.1	0	38.1
1	0.5	1	28
1	0.6	1	28
1	0.4	1	7.6
1	0.2	1	7.5
1	2.9	1	26.1
2	0.5	2	0
2	0.5	2	24.0
2	0.1	2	30.4
2	0.6	2	9.9
2	33.2	2	0.2
3	0.5	2	0.7
3	7.3	2	26.1
3	2.9	3	28
3	2.9	-	-

Appendix 4: Change in levels of *V. dahliae* in soil with time



Appendix 5. Crop diary: January 2006 - January 2007

10 January 2006 - Soil sampled to determine *V. dahliae* levels

5 May 2006 - Butisan S (2.5 L/ha) + Kerb (2.0 L/ha) applied to plots for weed control

26 April 2006 - Trees planted

20 July 2006 - Roundup (1.5 L/ha) + 2,4 D (1.5 L/ha) applied for weed control

11 October 2006 - Disease assessed and trees sampled (one third removed)

12 January 2007 - Soil sampled to determine *V. dahliae* levels