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GROWER SUMMARY

1. Headline

- Chloropicrin and metam sodium/dazomet were very effective at reducing levels of *V. dahliae* microsclerotia and stubby root nematode.
- BSD treatment moderately reduced numbers of *V. dahliae*.
- Sudan grass treatment was ineffective against both *V. dahliae* and nematodes.

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 was 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 be by 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 for the field-grown tree industry one or more alternatives to methyl bromide for soil disinfestation. The treatment should be applicable broadacre and provide effective control of *V. dahliae* to sufficient depth to enable economic production of a crop down 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 four 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

Site selection

In January – March 2005, soil samples were collected from six fields in Hampshire, considered from their recent cropping history to have a high risk of containing *V. dahliae*. The highest level of *V. dahliae* was found in Hangary field, Andlers Ash Nursery with 6.3 colony forming units/g soil. Previous crops grown on the field were potatoes (1988) and more recently (2000-2004) tagetes. *Acers* had never been grown on the land.

Soil treatments

A large plot (18.5 m x 10.8 m) experiment was established, with five replicates of five different 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 as a green manure. No soil cover.
3. Italian rye-grass cv. Danergo, grown on plots and incorporated on 9 August, at approximately 5.7 kg fresh weight/m², to 35 cm by spading machine, irrigated to field capacity, and covered with oxygen-impermeable plastic to create anaerobic conditions in the soil.
4. K&S Chlorofume (99.5% chloropicrin) injected to 35 cm depth at 40 mL/m^2 (maximum permitted rate) by contractor on 26 August.
5. Basamid (98% dazomet) applied at 45 g/m² and incorporated to 20 cm and Sistan 51 (51% metam sodium) injected at 25-35 depth at 90 mL/m^2 (= 46 mL/m^2 of metam sodium, maximum permitted rate) by contractor on 26 August.

Before treatments were applied each plot was sampled (50 cores) to a depth of 20 cm, in a grid pattern and the soil samples were tested for *V. dahliae* and nematodes. Levels of *V. dahliae* ranged from 4.9 to 38.2 propagules/g soil. The levels of *V. dahliae* found were used to determine the location of replicate blocks in the experiment. The five plots with the lowest levels of *Verticillium* (4.9-8.8 propagules/g) were block 1, through to the five with the highest levels (20.6-38.2 propagules/g) being block 5. Treatments within a block were allocated at random. Soil samples were retained and subsequently tested for *V. dahliae* levels using a PCR method.

The threshold levels of *V. dahliae* in soil, above which damage to tree species is likely to occur, are currently unknown. One of the objectives of this project is 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.

Effect of treatments

Control of *V. dahliae*

Levels of *V. dahliae* in soil were significantly reduced by treatment with Chlorofume, Basamid/Sistan 51 and BSD. The two chemical fumigants (95-97% reduction), were significantly more effective than the BSD treatment (36% reduction).

Levels of *V. dahliae* in untreated, fallow soil increased by 139% between March 2005 and January 2006. A range of levels of *V. dahliae* (0-62 propagules/g) is now present at the experimental site for determination of susceptibility of tree species to differing levels.

Control of nematodes

Treatment with Chlorofume, and a combined treatment of Basamid and Sistan 51, both significantly reduced the number of stubby root nematodes. Incorporation of Sudan grass and a BSD treatment using rye grass were ineffective.

Effect on tree growth

This project is continuing to determine the effect of the four pre-plant soil treatments on tree growth and occurrence of verticillium wilt in *Acer platanoides* ‘Emerald Queen’, *Tilia cordata* ‘Greenspire’ and *Sorbus aria* ‘Majestica’. Trees will be planted in 2006.

4. Financial benefits

In April 2005, discussion with nine UK tree growers with a total annual production of over 92,000 *Acers*, 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 is £364,933 and the annual production cost loss is £182,467. Identification of an effective method for soil disinfestation method is therefore likely to have substantial financial benefits for UK growers of verticillium susceptible trees.

5. Action points for growers

1. When selecting sites for production of verticillium-susceptible tree species, growers should be aware that both fat hen (*Chenopodium alba*) and marigold (*Tagetes erecta*) are hosts of *Verticillium dahliae*. Occurrence of significant levels of either of these species in a field may result in an increase in levels of *V. dahliae* in the soil, thereby increasing the risk of verticillium wilt in a following susceptible crop.

SCIENCE SECTION

1. Introduction

Methyl bromide has been the standard treatment for control of soil-borne *Verticillium dahliae* for many years due to its proven efficacy, broad-spectrum activity and reliability under different soil conditions. As this chemical will no longer be available for pre-plant soil treatment after 31 December 2006, an alternative is urgently required that reliably provides an acceptable level of soil disinfestation and is technically and economically feasible to use for the production of field grown trees in the UK. It is estimated that losses in the UK will exceed £150,000 annually if no suitable alternative is identified. The treatment must control *V. dahliae*, cause of Verticillium wilt, as this fungus is widespread in soils and causes a serious disease. The treatment should be applicable broadacre and provide effective control to sufficient depth (22) to enable economic production of a four-year-down crop. Previous studies have identified some currently available alternatives potentially suitable for use in the UK. Chloropicrin, metam-sodium/dazomet, biological soil disinfestation and a green manure crop of Sudan grass currently appear the most promising.

Seasonal variation in susceptibility to *V. dahliae* has been recorded in some species (1); it is therefore likely that a multi-year trial, as planned in this project, will better test the susceptibility of tree species than a short-term trial.

The methods used to quantify *V. dahliae* in soil can markedly influence the results; even different laboratories using the same testing procedure on a common soil sample sometimes produce significantly different results. Readers should therefore take greater note of the relative differences in *V. dahliae* levels in soil samples tested at a single laboratory, than of the absolute levels of propagules (microsclerotia) found.

Chemical alternatives

The main currently available chemical alternatives to methyl bromide for control of fungal pathogens are chloropicrin, dazomet and metam sodium.

Chloropicrin

Chloropicrin is included as our standard reference treatment against which the effectiveness of the three more novel treatments can be compared. Chloropicrin was shown to be capable of providing control of verticillium wilt in strawberry comparable to methyl bromide (13). This chemical is slightly heavier than air and therefore offers the prospect of good sterilisation to depth, though probably less so than methyl bromide.

Like all the soil sterilisation chemicals, chloropicrin will be reviewed as it comes up for re-registration under the EU review programme. Whether or not it remains available depends on the safety data package submitted, and whether the registration holder seeks to generate new data to answer any queries raised by the EU. It is interesting to note, and perhaps hopeful for the continued availability of chloropicrin, that several new products in the USA contain chloropicrin as a major active ingredient (e.g. Midas, Telone C35).

Dazomet

Dazomet is now being used by some strawberry growers for control of verticillium wilt. It gave good control of verticillium wilt in strawberries when used at 450 kg/ha (13). It has been shown to provide good weed control in tree and shrub seed beds (14), and some control of a range of fungal pathogens, including *Fusarium oxysporum* and *Rhizoctonia tuliparum* (O'Neill, unpublished). Dazomet is formulated as a prill (small granules) and needs to be incorporated thoroughly and evenly into the soil, to the depth to which sterilisation is required. The active gas methyl-isothiocyanate (MITC), released when the granules contact water, is lighter than air and moves upwards. Hence it is difficult to achieve a good result at depth. A high level of soil moisture (50-70% field capacity), a soil temperature >10°C and a fine, clod-free tilth, are required to achieve good results.

Metam sodium

Metam sodium is a liquid and can be injected at depth or applied in irrigation water to soil at field capacity and washed down to depth. Lateral movement occurs primarily by movement in soil moisture. Although probably better than dazomet for treating soil at depth (because of the ability to inject to a particular depth), experience suggests it is not as robust or as thorough a treatment as methyl bromide. A combined dazomet and metam sodium contractor-applied treatment is available (9). This seeks to make the most of these two chemicals by incorporating dazomet in the surface layer and injecting metam sodium at 20-25 cm depth into the cavity created by tines. Good results against a range of fungi were obtained (15), including *V. dahliae* in one test. In Oregon, USA, metam sodium applied to the soil surface at 93 mL/m^2 in 500 mL/m^2 of water, and rotovated-in (depth unspecified), resulted in a significant decline in *V. dahliae* at 5, 10, 20 and 30 cm depths (10). At 10 cm, microsclerotial numbers were reduced from 115 to 2.3 cfu/g; at 20 cm from 171 to 7.3 cfu/g. Treatment with metam sodium at 23 mL/m^2 was less effective but still gave large and statistically significant reductions (e.g. from 171 to 48 cfu/g at 20 cm). The maximum permitted application rate in the UK is 90 mL/m^2 of Sistan 51 (46 mL/m^2 of metam-sodium).

Biological alternatives

Two promising biological methods are biological soil disinfestation (BSD) (7,19), and Sudan grass (*Sorghum vulgare* var. *sudanense*) (12, 18). Neither method has been thoroughly tested experimentally in the UK; soil, climatic and other differences may result in different efficacy here compared with their countries of development (the Netherlands and the USA). The ability to grow Sudan grass in the UK was demonstrated recently, but plots were unreplicated and effects on *V. dahliae* were inconclusive.

Biological Soil Disinfestation (BSD)

BSD involves incorporation of fresh organic material (e.g. Italian rye-grass) into soil and mulching with airtight plastic. This combination creates an anaerobic condition that results in pathogen elimination. The mode of action is believed, in part, to be generation of fungitoxic volatile fatty acids, such as acetic acid. Long-term studies in the Netherlands (7, 8) have demonstrated BSD to be an effective alternative to methyl bromide for reducing soil inoculum levels of a wide range of plant pathogenic soilborne fungi and nematodes (except *Pythium*). In a 4-year study on verticillium wilt in trees at two sites, on land artificially infested with *V. dahliae*, soil inoculum levels were reduced by 85% after BSD and did not increase for 4 years. Populations of *Pratylenchus fallax* (a nematode known in Holland for its interaction with *V. dahliae*) were reduced by 95-99%. The incidence of infection by *V. dahliae* in *Acer platanoides* and *Catalpa bignonioides* was reduced by 80-90%. BSD is currently practiced by farmers in the Netherlands to control soilborne pests and diseases in strawberry, asparagus and woody ornamentals (19).

Sudan grass

Numerous different varieties of Sudan grass and sorghum-Sudan grass hybrids are described. They are annual warm season grasses that are tolerant of heat and drought, but are killed by the first hard frosts. They are used as a cover crop to suppress weed growth, improve soil quality, store residual soil nitrogen, reduce soil compaction (through development of an extensive root system) and to increase organic matter levels in the soil.

Some varieties produce high levels of dhurrin, a precursor of hydrogen cyanide (HCN). High dhurrin cultivars were reported in Canada to suppress nematodes. The top growth can be used as a food for livestock providing the variety is one that does not produce high levels of HCN, and/or measures are taken to prevent grazing when HCN levels are high (25). Presumably varieties bred with low dhurrin content for animal feed are less suitable for use as pre-plant soil treatments to control nematodes and fungal pathogens.

The mode of action of Sudan grass in suppressing verticillium wilt is very different from that of BSD. The exact mechanism is unclear, with evidence for both chemical and biological mechanisms. After growing for 3-6 months, the foliage is chopped and incorporated into soil where fungitoxic hydrogen cyanide and other secondary metabolites are released. Disease reduction using Sudan grass has also been correlated with major change in microbial populations in the soil, particularly increases of the non-pathogenic *Fusarium equiseti* (i.e. possibly indicating biological control).

The occurrence of verticillium wilt in potato in Florida was observed to decline coincident with the introduction of sorghum-Sudan grass as a cover crop (12). In Washington, verticillium wilt was suppressed and potato yield increased after pre-cropping with a pea-Sudan grass rotation during the same year (20). In a series of three field studies comparing Sudan grass to fallow or barley before a potato crop (18), the resulting wilt incidence was reduced by i) a green manure crop of Sudan grass for one season, ii) growing Sudan grass followed by barley, iii) when Sudan grass cuttings were added to fallow plots and incorporated or iv) when Sudan grass was grown, cut and removed during the season before potatoes. Thus, there is evidence of an effect from both foliage and roots. Yields were negatively correlated with wilt incidence.

In another study, a green manure crop of *Sorghum vulgare* var. *sudanense* cv. Monarch gave better control of verticillium wilt in potato than pea, rape, corn and rye green manures, and increased potato yield (12). Over a 4-year period, *V. dahliae* in fallow soil remained at around 40 cfu/g, whereas under Sudan grass cropped annually for 3 years the inoculum level fell from 60 to around 10 cfu/g. In trials, a reduction in verticillium wilt in potato, from around 50% to 20% plants affected, was observed after Sudan grass even when no reduction in microsclerotial numbers was found.

Not all studies with Sudan grass, however, have shown an effect on the *V. dahliae* or wilt. Work on a silty-clay loam in the USA, using microsclerotia buried in bags, found that a green manure crop of Sudan grass did not reduce *V. dahliae* populations in soil, although metam sodium did (10). Another study found that cropping with Sudan grass for 1 or 2 years did not reduce verticillium wilt incidence in subsequent potato crops (11); however, *V. dahliae* levels in this study were extremely low, reducing opportunity to detect significant effects. The grass was not incorporated until November, which may have reduced efficacy.

In other studies, Sudan grass was shown to be antagonistic to *Meloidogyne hapla* nematodes (21). Other work has shown that Sudan grass can harbour high densities of stem lesion nematodes (*Pratylenchus penetrans*).

If mown when 1-1.5 m tall, the root system of Sudan grass is increased considerably, and managed in this way as a cover crop it is good at loosening compacted soils. Possibly this effect on the soil allows better root development in subsequent crops and consequently reduces the impact of verticillium wilt.

Steaming and other alternatives

Other recent studies on soil disinfestation include plate steaming (16), lifting soil to steam it before relaying it (17), and the incorporation of brassica residues or mustard pellets to generate MITC. Work on both the above methods is on-going in a Horticulture LINK (HL0177) and other projects.

Testing soil for *V. dahliae*

Development of a soil test for *V. dahliae,* based on plating soil onto a selective agar medium, and its use to predict risk of verticillium wilt of strawberry has been published (2-5). Work on validation of a PCR method for testing soils for verticillium wilt of strawberry commenced in 2005 (HDC project SF 70).

The soil-plating test is now widely used in the UK to aid decisions on whether a particular field is suitable for growing verticillium-susceptible strawberry varieties. It is offered as a commercial service by ADAS (at ADAS High Mowthorpe) and by EMR Ltd (at East Malling). A disadvantage of the current agar-plating test is that it takes a minimum of 6 weeks. Molecular methods based on DNA detection, such as the Polymerase Chain Reaction (PCR) offer the possibility of a very sensitive and very specific test that takes only a few days. Work will also be needed to determine an appropriate soil sample size and to understand how results relate to disease risk. It is possible that a PCR test may detect conidia and mycelium of *V. dahliae* as well as microsclerotia.

With different strawberry varieties, a body of evidence has developed from soil-plating tests and grower observations to allow critical threshold levels to be set. When the level of *V. dahliae* in a soil exceeds a threshold, growers are advised to grow a resistant variety or to disinfest the soil. Although experimental results from Holland and grower observation indicate that different tree species and varieties differ in their sensitivity to *V. dahliae* (6, 26), threshold levels have not been set using the UK soil testing procedure. It is therefore difficult to interpret soil test results in order to aid decision – making on tree planting, unlike strawberries where evidence-based advice can be given. This project will investigate the response of four different tree species to five different levels of *V. dahliae* in the soil.

Types of *V. dahliae*

Recent studies in Holland showed that isolates belonging to each of the two major compatibility groups of *V. dahliae* occurring in the Netherlands (VCG-NL1 and NL2) caused severe symptoms of wilt in most of the susceptible woody ornamentals tested (*Acer campestre, Acer platanoides, Acer pseudoplatanus, Catalpa bignonioides, Cotinus coggyria, Robina pseudoacacia, Rosa canina* and *Tilia cordata*) (6). Disease progress differed between plant species but was generally the same for the two VCGs. These findings imply that VCG identification does not contribute to disease prediction in these woody ornamentals.

2. Materials and methods

Site selection

In January and March 2005, soil samples were taken from fields at Hillier's Andlers Ash Nursery, Hants, and their nearby Gatehouse site. Samples consisted of 75 cores, taken to a depth of 20 cm. The samples were sent to ADAS High Mowthorpe for analysis for *V. dahliae* and nematode numbers. Initially two fields were sampled in January 2005. As only low levels of *V. dahliae* were found, four further sites were sampled. Four of the soils were also tested at East Malling Research, using both a rapid (two-week) and conventional soil-plating methods. A soil sample was also tested for texture, pH and nutrient status.

Experiment design

Treatments were arranged in a randomised block, split-split plot design with 5 replicates of 5 treatments (i.e. 25 main plots). Main plots were soil treatments, sub-plots are replicates (15 within main plot) and sub-sub-plots are tree species (4 species). A plan of the experimental lay out is shown in Appendix 1.

The plots will be planted with trees in 2006. Each sub-sub-plot will contain 3 replicate plants of one tree species grown adjacent to each other. Each sub-plot will contain one replicate of each sub-sub-plot (i.e. 3 plants of each of the 4 tree species). The different species will be arranged in a randomised order. This will give a total of 180 trees per plot at the start of the experiment. Numbers will be reduced to 120 trees at the end of growing season 1, and to 60 trees at the end of growing season 2, by removal of two plants from each sub-sub-plot for destructive assessments of verticillium wilt.

The treated soils will be planted with two species reported to be highly susceptible to *V. dahliae* and one species (*Sorbus aria*) that is not:

Acer platanoides \`Emerald Queen\`
Tilia cordata \`Greenspire\`
Sorbus aria \`Majestica\` on *S. intermedia* rootstock

Based on tree numbers and spacing requirements, the main plot size is 10.8 m wide x 23 m long. This will comprise 5 experimental rows and a single guard row of *Betula pendula* around the edges (i.e. a total of 7 rows, 1.8 m apart). Seedlings will be planted at greater than usual density within rows (40 cm apart). At the end of growing season 1, the first seedling in each run of 3 will be removed. At the end of growing season 2, the last tree in each run of 3 will be removed. From around 20 months after planting (2 growing seasons), trees will thus be 1.2 m apart.

A lead-in/pull out length of 5 m was allowed at the end each plot to accommodate start-up and pull-out of chemical application equipment.

Pre-plant soil treatments

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	Chloropicrin 99.5%	Injected to 35 cm 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	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	

Baseline *V. dahliae* levels in the soil and allocation of treatments to plots

Soil samples were taken from each plot on 6 April 2005 and tested for *V. dahliae*. A grid pattern was used with the plot being divided into 25 squares (5x5), 2 cores were then taken from each square giving a total of 50 cores taken, to a depth of 20 cm. Soil samples were sent to ADAS High Mowthorpe, for laboratory analyses of *Verticillium* propagules per gram of soil using the standard agar-plating method. Sub-samples were sent to EMR for testing by PCR. Plots were listed in order of how many propagules there were per gram of soil. The list was divided into five blocks, block 1 having the five lowest propagule numbers, through to block 5 having the five highest propagule numbers. A randomisation was done to apply the treatments within a block.

Application of treatments

The whole trial area had grown *Tagetes* in 2004. The crop remains were ploughed in on 25 May 2005. The sequence of operations for application of each pre-plant treatment are given below.

Treatment 1 – Untreated control

These plots were maintained as bare ground. Power harrowed 02/08/05.

Treatment 2 – Sudan grass (Sorghum vulgare *var* sudanense *cv. Nigrum*)

- 30/05/05 - Sudan grass drilled at 3.5 kg/ha, using a 3 m air drill.
- 06/09/05 - Sudan grass assessed for dry matter content – fresh weight 3 kg/m^2, dry weight 0.9 kg/m^2. Soil water content as % field capacity (FC) = 50.5%. Fat hen present in all plots.
- 07/09/05 - Cut with front mounted mower, ploughed to 20 cm incorporating 80% of material, power harrowed to complete incorporation and rolled to seal the plot. Soil at 50.5% FC, temperature 20°C.
- Lab analysis of Sudan grass showed it to have a C:N ratio of 64, a total nitrogen content of 0.86 g/100 g, and an oven dry matter of 32.3%.

Treatment 3 – Biological soil disinfestation (BSD)

- 30/05/05 – Italian tetraploid rye grass (cv. Danergo) drilled at 50 kg/ha using 3m air-drill.
- 27/06/05 - Sprayed with Starane at 0.5L/ha, to control fat hen and other weeds. Treatment had little effect upon the fat hen, but showed some phytotoxic effects upon the rye-grass.
- 02/08/05 – Rye grass sampled for green matter (2.3 kg/ m^2 rye grass fresh weight), 1 kg/ m^2 fat hen. Soil moisture = 82% FC.
- 09/08/05 – Rye-grass cut from area outside plots and spread equally over all the plots to increase the green matter for incorporation to 5.7 kg/ m^2. Each plot was flooded with 3 loads of water from a 5000 L bowser, equivalent to 75 mm/cm^2. Once flooded, plots were spaded to a depth of 30 cm using an Imants spader. Virtually impermeable film (VIF), was used to cover the plots. All edges and joins were hand-buried to a depth of 20 cm. Three spans of VIF were used resulting in a total covered area of approximately 9 m wide.
- 26/08/05 – soil temperature under the plastic was 20°C, compared with 14°C in untreated uncovered soil at 9:00 am. Some small holes noticed in VIF as a result of the fat hen stems pressing on the plastic. These were sealed with tape.
- 06/09/05 – soil temperature under plastic 27°C, compared with 20°C in untreated uncovered soil at 1:45pm.

- 28/09/05 – VIF on one plot cut and holes dug to 45 cm. No smell of decomposition although some small patches of blackened vegetation seen. Soil moist but not waterlogged. Soil did not look anaerobic. Holes patched and re-sealed to protect soil overwinter. Rye-grass starting to re-grow under plastic.

Treatment 4 – Chloropicrin

- 02/08/05 – plots power harrowed to remove fat hen and other weeds.
- 26/08/05 - K&S Fumigation Ltd injected Chlorofume (99.5% chloropicrin) to 35 cm depth at 40 mL/m^2 (maximum permitted rate). The machine used was built by K&S to designs similar to those used in North America and Europe. Gas tanks are held on the front of the tractor, compressed using nitrogen and then the chloropicrin passes along a series of tubes to a set of hollow tines at the rear of the tractor. Flow meters in the pipes measure and control the amount of gas applied. Five widths of LDPE sheeting (35 µm thick) were used to cover the plots to a width of 10.6 m, immediately after injection. All the sides were buried whilst the edges that overlapped were glued. Soil at 64% FC, temperature at 20cm 14°C.

Treatment 5 – Basamid / Sistan 51

- 02/08/05 – plots power harrowed to control fat hen and other weeds.
- 26/08/05 - K&S Fumigation Ltd applied Sistan 51 (51% metam sodium) at 90 mL/m^2 (= 46 mL/m^2 of metam sodium) using a Rumpstadt to a depth of 20 cm. This was then spaded in to a depth of 30 cm using an Imants spader. The Basamid (98% dazomet) at 45 g/m^2 was applied to the surface of the spaded soil using a purpose-built front-mounted spreader. This consists of a series of containers mounted on the front of the tractor, each containing an equal amount of Basamid. The granules are released at a set rate, controlled by a land wheel. Once on the soil surface the Basamid was then power harrowed to a depth of 20 cm. The surface of the plots was smeared with a roller, providing a seal to the gases being released. Once the chemicals had been applied to all plots, a plastic-laying machine was then used to cover the plots. Five widths of LDPE (35µm thick) were used to cover the plots to a width of 10.6 m. All the sides were buried whilst the edges that overlapped were glued.

Measurement of soil moisture

Ten soil cores were taken, 0-20 cm depth immediately prior to pre-plant soil treatments. The field capacity (%FC) was determine using the formula: % FC = Field Weight - Dry weight/ (Drained wet weight – Dry weight). To determine dry weight, samples were dried overnight in an oven at 110°C. To determine drained wet weight, samples were wetted to saturation, excess allowed to drain away, and weighed.

Determination of *V. dahliae* in soil by Polymerase Chain Reaction (PCR)

As part of HDC project SF 70, development of a real-time PCR test for *V. dahliae* has examined the extraction of propagules from soil, extraction of DNA from propagules and subsequent PCR.

Although progress has been made in these areas, there are still a few outstanding issues to address. These relate to confirmation of PCR primer specificity, and possibly improvement of DNA extraction/PCR efficiency. Once a suitably robust protocol has been defined, soils currently in storage will be processed.

3. Results and Discussion

Site selection

Out of six fields tested in spring 2005, Hangary Field at Andlers Ash Nursery was found to have the greatest level of *V. dahliae* (Table 3.1), and was therefore chosen as the experimental site.

Table 3.1 Level of *V. dahliae* and nematodes in potential trial site fields, spring 2005.

Field identification	Date sampled	*V. dahliae* (propagules/g soil)	Nematode numbers/L soil					
			Stubby root	Stunt spiral	Cyst juveniles	Root lesion	Needle	Dagger
1. Alders	24 Jan	0.6	475	1275	0	0	45	0
2. Gatehouse	24 Jan	0.2	275	1150	0	275	5	0
3. Gatehouse 1	2 Mar	3.4	-	-	-	-	-	-
4. Gatehouse 4	2 Mar	4.6	-	-	-	-	-	-
5. Gatehouse	2 Mar	0.1	-	-	-	-	-	-
6. Hangary	2 Mar	6.3	-	-	-	-	-	-

In testing at EMR, a two-week 'whole-soil' test, in which the soil sieving stage is not included in sample preparation, failed to detect *V. dahliae* in any of four samples tested (samples 3-6). However, a conventional six-week test on the Hangary samples confirmed a relatively high level of *V. dahliae*, at 11.0 propagules/g soil.

Soil type

The soil at Hangary field was a loamy sand, pH 7.2, with 1.9% organic matter. Levels of major nutrient in April 2005 were: P, 45 mg/L (index 3); K, 35 mg/L (index 1), Mg, 35 mg/L (index 1) and NO_3, 5 mg/L (index 0).

Levels of *V. dahliae* and nematodes in spring 2005

Levels of *V. dahliae* and nematodes in the individual plots at Hangary Field are shown in Table 3.2. Baseline levels of *V. dahliae* as determined by the PCR method are not yet available. Pre-plant soil treatment allocation to individual plots was therefore made using the soil-plating results alone.

Table 3.2 Levels of *V. dahliae* and nematodes in individual plots at Hangary Field – April 2005.

Plot	*V. dahliae* (propagules/g soil)	Nematode numbers/L soil					
		Stubby root	Stunt spiral	Cyst juveniles	Root lesion	Needle	Dagger
1	7.0	525	0	0	0	0	0
2	11.7	400	0	0	0	0	0
3	9.3	350	0	0	0	0	0
4	9.3	225	0	0	0	0	0
5	11.3	475	0	0	0	0	0
6	4.9	550	0	0	0	0	0
7	9.5	550	0	0	0	0	0
8	7.5	325	0	0	0	0	0
9	10.8	325	0	0	0	0	0
10	10.7	425	0	0	0	0	0
11	8.8	200	0	0	0	15	0
12	10.9	150	0	0	0	0	0
13	8.0	300	0	0	0	0	0
14	14.0	150	0	0	0	0	0
15	31.4	200	0	0	0	0	0
16	19.5	275	0	0	0	5	0
17	20.6	275	0	0	0	0	0
18	16.8	325	0	0	0	0	0
19	17.8	250	0	0	0	5	0
20	38.2	450	0	0	0	5	0
21	26.3	275	0	0	0	0	0
22	13.4	375	0	0	0	0	0
23	9.7	425	0	0	0	0	0
24	17.4	625	0	0	0	0	0
25	32.6	400	0	0	0	0	0
Mean	15.1						

Levels of *V. dahliae* ranged from 4.9 to 38.2 propagules/g. The mean level for all 25 plots was 15.1 propagules/g, greater than that recorded by ADAS (6.3) or EMR (11.0) on a single soil sample taken across all plots. These results illustrate the variability and patchiness in *V. dahliae* levels that can occur across a field. Although adjacent plots sometimes had similar levels of *V. dahliae* (e.g. plots 15, 20 and 25; see Appendix 1), in other areas adjacent plots differed greatly. Overall, the greater levels of *V. dahliae* occurred at the lower, western edge of the site. For blocking of soil treatments according to *V. dahliae* soil infestation, the differentiating levels were:

Block 1 (lowest levels)	4.9 – 8.8 propagules/g soil
Block 2	9.3 – 10.7
Block 3	10.8 – 13.4
Block 4	14.0 – 19.5
Block 5 (highest levels)	20.6 – 38.2

For a comparison, the levels of *V. dahliae* recorded in 362 soil samples, tested by the same method at the same laboratory from fields across England and Wales between October 2003 and September 2005, are tabulated below. These samples were submitted as possible sites for strawberry production. Levels ranged from nil to 286 propagules/g soil, with *V. dahliae* detected in 88% of samples. Forty-one percent of soils had >5.0 propagules/g, and 26% had >10.0 propagules/g. A mean field level of 6.3 propagules/g is therefore in the higher 40% of samples.

Table 3.3 Frequency of soil samples with different levels of *V. dahliae* (propagules/g soil) in samples received by ADAS, October 2003- September 2005.

% in each category (*V. dahliae* propagules /g soil)							
Samples	Nil	0.1-1.0	1.1-5.0	5.1-10.0	10.1-20	21 -50	>50
Number (n=362)	45	101	66	56	41	32	21
%	12.4	27.9	18.2	15.4	11.3	8.8	5.8
Cumulative %	12.4	40.3	58.5	73.9	85.2	94.0	99.8

The nematodes found were stubby root (*Trichodorus* spp.), at low-moderate levels in all plots, stunt spiral (*Tylenchorhynchus* spp.) at low levels in two plots and needle (*Longidorus* spp.) at low levels in two plots. Advice from the project entomologist was that these numbers and species pose no immediate risk to trees. No root lesion (*Pratylenchus* spp.) or dagger nematodes (*Xiphinema* spp.) were found. The previous crop on site was *Tagetes*. Work in another HDC project (BOF 50), found that *Tagetes* greatly reduced numbers of *Pratylenchus* (albeit initial numbers were very low) but did not affect other nematode species. The *Tagetes* crop here may explain the lack of *Pratylenchus* on this site.

Weed control

The broad-leaf herbicide (Starane) used to control weeds in rye-grass and Sudan grass plots, failed to control fat hen satisfactorily. This was the major weed that developed on the site. No woody nightshade, a potential major host of *V. dahliae*, was observed.

Other plots were maintained weed free by cultivation.

There have been occasional reports of *V. dahliae* occurring in fat hen (23). Samples were collected from the rye-grass plots on 3 August and examined in the laboratory. No vascular staining was observed and no microsclerotia were found in the roots. Stem bases sections were tested for latent *V. dahliae* by a paraquat treatment and incubation method (24). *V. dahliae* was detected in 3 out of 15 stem sections. Identification was confirmed by a PCR test specific for *V. dahliae* (V Krishnamurthy, pers. comm.). Subsequent discussion with researchers in Holland indicated that fat hen should not result in a bulking-up of *V. dahliae* in soil providing the weeds are cut while still green. This was the case in this trial. Microsclerotia form on stem, leaves and roots when the plant dies and dries out. When fat hen is present in a BSD treatment, the green plant remains become a food source for other flora and fauna and multiplication of *V. dahliae* is considered unlikely (J. Lamers, pers. comm.). Based on this, and the difficulty of hand weeding 10 large plots, it was decided not to attempt hand removal of fat hen prior to treatment application.

Soil conditions at time of pre-plant soil treatment

Soil temperature and moisture at the time of treatment applications are summarised in Table 3.4.

Table 3.4 Soil temperature and moisture and application of pre-plant soil treatments

Treatment	Date applied	Temperature (°C at 20 cm depth)	Soil moisture (% FC)
1. Untreated	-		
2. Sudan grass	Incorporated 6 Sep	20°C at 2pm	50.5
3. BSD	Incorporated 9 Aug	16°C at 10am	82.0*
4. Chlorofume	Injected 26 Aug$^+$	14°C at 9am	64.0
5. Basamid/Sistan 51	Applied 26 Aug$^+$	14°C at 9am	64.0

* Before application of 75 mm of water
$^+$ 75 mm rain occurred in the period 6 – 16 August.

<u>Sudan grass</u>

No definitive information was found on varieties most suitable for suppression of soil-borne pests and pathogens.

The variety *Nigrum* is reported to be grown in Holland for soil improvement and has been grown successfully at Andlers Ash. In a list of Australian varieties categorised by HCN content, cv. Nigrum was not listed. This variety was therefore chosen for use in this project. The root system of Sudan grass is reported to penetrate deeply into the soil when the crop is mown. There was insufficient growth by 2 months after drilling (early August) to risk cutting down the crop and still achieve a good quantity of young re-growth for incorporation into the soil. The Sudan grass was incorporated into the soil in early September, when soil temperatures were still warm (20°C at 2pm), in order to facilitate decomposition of the grass prior to planting with trees in 2006.

<u>BSD</u>

Experiments in Holland reported good control of *V. dahliae* and a reduction of verticillium wilt in subsequent tree species when soil was subjected to BSD with incorporation of 4 – 6 kg/m^3 fresh weight of green biomass. The biomass should be material that will easily decompose in soil, such as grass. If grass is sown in the autumn, it can then be mown in the spring to encourage flowering, the stems of which add greatly to the biomass. If grass is sown in the spring, when mown in late summer it consists primarily of leaves, with a lower organic matter content than that of stem. Researchers in Holland suggested that the exact type of biomass was not critical, several types have been shown to work; and that it should be incorporated in summer, when soil temperatures are high, for best effect. If incorporation is done in the summer, BSD can be restricted to just 6 weeks. In late autumn, BSD was not effective.

As this project started in April 2005, in order to be able to apply a BSD treatment in 2005 we had to work with spring-sown rye-grass. A tetraploid species was drilled at a high seed rate, and extra plots for grass production were grown adjacent to the trial area, in order to ensure a total fresh weight of at least 4 kg/m^2 was achieved. In practice, 2.3 kg of rye-grass + 1 kg of fat hen was grown on the plots and a further 2.4 kg of rye-grass was added from adjacent plots, to give a total of 5.7 kg/m^2. This was incorporated on 9 August when the soil temperature at 10am was 16°C at 20 cm depth.

Following discussion with Dutch researchers, plots were covered with VIF film, which was reported to be as good as ensilage plastic in excluding air. In practice, some small holes occurred where fat hen stems penetrated the film. Also, re-growth of grass beneath the clear plastic was visible from around 7 weeks after incorporation and continued until late October.

The plots were not rolled after incorporation of grass and irrigation and prior to covering with VIF because of the inability of machinery to travel over the wet soil.

When soil temperatures were recorded at 19 and 30 days after incorporation, it was 6-7°C greater beneath the plastic (27°C) compared with untreated, uncovered soil. This temperature increase may have resulted from covering with the VIF film and/or the heat generated from grass decomposition.

Chloropicrin

Treatment was applied on 26 August when soil temperature at 9am was 14°C, well above the minimum requirement of 10°C.

Basamid and Sistan 51

Treatment was applied in 26 August. Around 75 mm of rain had occurred on the farm in the period 10 – 16 August and soil was still at a high moisture content (64% FC) when the chemicals were applied. The LDPE covers were applied within around 1hr of the start of Basamid incorporation.

The covers on the BSD, Chlorofume and Basamid/ Sistan 51 plots were removed in January 2006 prior to soil sampling.

Effect of pre-plant soil treatments on *V. dahliae*, nematodes and weeds

Soil samples were collected from each plot on 10 January 2006 for determination of *V. dahliae* and nematode levels. Levels of *V. dahliae* were determined by the conventional plate test; sub/samples are stored for subsequent testing by PCR when this method has been validated.

Verticillium dahliae

There were no significant differences between treatments in the levels of *V. dahliae* detected in soil samples collected in March 2005, prior to treatment application. Mean levels ranged from 13.6 to 16.1 cfu/g (Table 3.5).

After soil disinfestation had been applied, the levels of *V. dahliae* differed significantly ($P<0.001$) between treatments. Mean levels were greatest in untreated soil (33.8 cfu/g) and were significantly reduced by Chlorofume (0.6 cfu/g), Basamid/Sistan 51 (0.7 cfu/g), and BSD (26.1 cfu/g). The two chemical fumigants were significantly better than the BSD treatment. Levels of *V. dahliae* following incorporation of Sudan grass (23.0 cfu/g) were not significantly different from the untreated control (33.8 cfu/g).

Expressed as a percentage reduction of the pre-treatment levels, treatment efficacy was: Basamid/Sistan 51, 97%; Chlorofume, 95%, Sudan grass 36%.

Interestingly, levels of *V. dahliae* increased in four of the five the untreated plots and in four of the five Sudan grass plots. Mean increases were 139% in untreated soil and 69% in the Sudan grass soil. Possibly these increases resulted from decay of verticillium-infested stem debris from the previous tagetes crop, releasing *V. dahliae* microsclerotia into the soil; *Tagetes erecta* is recorded as a host of *V. dahliae* (27). Increases in soil levels of *V. dahliae* microsclerotia are known to occur following some potato crops due to decay of verticillium-infested potato stem debris and release of microsclerotia.

Table 3.5 Effect of pre-plant soil treatments on levels of *V. dahliae* in soil as determined by conventional plate tests (cfu/g).

Treatment	Initial levels (March 2005)			Post-treatment levels (Jan 2006)		
	Mean	Min	Max	Mean	Min	Max
1. Untreated	16.0	7.0	32.6	33.8	7.6	62.2
2. Sudan grass	15.5	8.8	31.4	23.0	7.3	36.4
3. BSD	16.1	8.0	38.2	10.1	2.9	26.1
4. Chlorofume	14.3	4.9	26.3	0.6	0.1	0.6
5. Basamid/Sistan 51	13.6	7.5	20.6	0.3	0.2	0.7
Significance (16 df)	0.697			<0.001		
SED	2.138			6.35		

<u>Nematodes</u>

Stubby root nematodes were selected for analysis because a) these nematodes are capable of causing crop damage and b) these are the only nematodes that were present in significant numbers.

Statistical analysis confirmed that there were no significant differences between the numbers of stubby root nematodes in any of the five treatments when these numbers were assessed pre-treatment in March 2005 (P<0.05) (Table 3.6). However, when re-assessed after treatment and the passage of one growing season, in January 2006, differences in stubby root nematode populations were obvious (Table 3.6). Statistical analysis has confirmed that the populations of nematodes in the chloropicrin-treated plots and in the dazomet/metam sodium-treated plots were significantly lower than those in the remaining plots ($P<0.05$). There was however no significant differences between the number of stubby root nematodes in the untreated plots and those treated by incorporation of Sudan grass or by biological soil disinfestation with rye-grass.

It is possible to conclude, therefore, that neither the Sudan grass treatment nor the biological soil disinfestation had any effect on stubby root nematodes. In contrast, the chloropicrin treatment and the dazomet/metam sodium treatment seem to have been very effective at reducing the number of these nematodes in the soil.

The overall mean number of stubby-root nematodes in the soil pre-treatment was 353/litre. At this level, these nematodes would not normally be responsible for significant crop damage under UK conditions.

Table 3.6 Effect of pre-plant soil treatments on levels of stubby root nematodes in soil (nematodes/litre).

Treatment	Initial levels (March 2005)			Post-treatment levels (Jan 2006)		
	Mean	Min	Max	Mean	Min	Max
1. Untreated	380	250	525	350	150	650
2. Sudan grass	285	150	550	370	175	850
3. BSD	330	150	450	295	50	525
4. Chlorofume	430	225	635	0	0	0
5. Basamid/Sistan 51	340	275	400	0	0	0

<u>Weeds</u>

At removal of the polythene soil covers on 10 October, a comparison with the untreated plots indicated good weed control from the chloropicrin, Basamid plus Sistan 51 and the Sudan grass treatments. Effect of the BSD treatment on weed control was difficult to judge because of rye grass re-growth.

The untreated plots (which remained uncovered) had 90% ground cover from weeds, all low growing weeds less than 5 cm tall. These were mainly annual meadow grass, chickweed, mouse-ear chickweed and a few thistle rosettes.

The chloropicrin-treated plots were relatively weed-free with an occasional annual meadow grass and willow herb sp., less than 1 per m^2, where covers had remained down. A few more grass weeds were present where covers had blown off. The exception was plot 21, which had remained well-covered with polythene, where an even mat of annual meadow grass was growing in the middle of the three treatment strips, indicating the chemical may not have been injected here.

The Basamid-treated plots were also relatively free of weeds, with occasional grass and willowherb seedling present. Green and orange algae/mosses were present on the surface of the soil.

In the Sudan grass-treated plots, there were low levels of grass weeds; the plots appeared reasonably clean and tidy.

In the BSD-treated plots, there was 100% ground cover with either rye grass tussocks (20 cm high) or annual meadow grass. There was more annual meadow grass towards the centres of plots, where plants look yellow and in places were mouldy. Occasionally during soil sampling a slightly rotten smell was present, although the soil samples did not look any different to those from other plots. An exception was plot 9, where no rotten smell was noted and there was abundant rye- grass re-growth.

All the covers had holes in, especially those over the BSD plots (VIF film). The covers on plots 4 and 5 had virtually blown off along one side and some of the other covers were loose and flapping on the chemical-treated plots.

Heavy rains had caused erosion across parts of the site. Plot 23 had a large trench (c. 30-cm deep) that had been carved through the outer edge of it by rainwater that had passed under the covers.

4. Conclusions

1. In autumn 2005, treatment of a loamy sand soil with Chlorofume at 40 ml/m^2, and a combined treatment of Basamid at 45 g/m^2 and Sistan 51 at 90 ml/m^2, both significantly reduced the number of stubby root nematodes. Incorporation of Sudan grass (3 kg/m^2), and a BSD treatment using rye grass (5.7 kg/m^2), were ineffective.

2. Chlorofume, Basamid/Sistan 51 and BSD all significantly reduced levels of

V. dahliae microsclerotia in soil. The two chemical fumigants (95-97% reduction), were significantly more effective than the BSD treatment (36% reduction).

3. Levels of *V. dahliae* in untreated fallow soil increased by 139% between March 2005 and January 2006.

4. A wide range of levels of *V. dahliae* (0-62 cfu/g) is now available at the experimental site to determine the susceptibility of tree species to differing levels.

5. Fat hen (*Chenopodium alba*) was confirmed as a host of *V. dahliae*.

5. Technology transfer

Meetings

- Project steering group meeting, Andlers Ash, Hants,12 May 2005
- Project steering group meeting, East Malling, 21 February 2006

Presentations

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

6. Acknowledgments

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APPENDIX 1

	Plot 1 Block 1 Treat 1 Vert 7	Plot 6 Block 1 Treat 4 Vert 4.9
	Plot 2 Block 3 Treat 5 Vert 11.7	Plot 7 Block 2 Treat 2 Vert 9.5
	Plot 3 Block 2 Treat 1 Vert 9.3	Plot 8 Block 1 Treat 5 Vert 7.5
	Plot 4 Block 2 Treat 4 Vert 9.3	Plot 9 Block 3 Treat 3 Vert 10.8
	Plot 5 Block 3 Treat 4 Vert 11.3	Plot 10 Block 2 Treat 5 Vert 10.7
Plot 11 Block 1 Treat 2 Vert 8.8	Plot 16 Block 4 Treat 5 Vert 19.5	Plot 21 Block 5 Treat 4 Vert 26.3
Plot 12 Block 3 Treat 2 Vert 10.9	Plot 17 Block 5 Treat 5 Vert 20.6	Plot 22 Block 3 Treat 1 Vert 13.4
Plot 13 Block 1 Treat 3 Vert 8	Plot 18 Block 4 Treat 2 Vert 16.8	Plot 23 Block 2 Treat 3 Vert 9.7
Plot 14 Block 4 Treat 3 Vert 14	Plot 19 Block 4 Treat 1 Vert 17.8	Plot 24 Block 4 Treat 4 Vert 17.4
Plot 15 Block 5 Treat 2 Vert 31.4	Plot 20 Block 5 Treat 3 Vert 38.2	Plot 25 Block 5 Treat 1 Vert 32.6

Trial: Verticillium wilt in trees (HNS 137)

Date: 2005

Basic trial plan, showing blocking and treatments – with initial verticillium levels(before soil treatment) also shown (number of propagules/g of soil).

Treatments

1. Untreated – yellow
2. Sudan grass – green
3. Biological soil disinfestation – pink
4. Chlorofume – blue
5. Basamid/Sistan 51 – red

Note that allocation of treatments to plots was done on the basis of levels of V. dahliae in the soil.

This ensured that each treatment was tested at a range of Verticillium levels in the soil.

Each plot is 12.6m wide x 22.6m long

To farm

APPENDIX II

Trial view

Figure 1 - Chloropicrin application

Figure 2 - Plough and mower used for incorporating Sudan grass

Figure 3 - Basamid application, Rumpstadt attached as well