Using Soil *Verticillium dahliae* Infestation Data to Determine Risk of Verticillium Wilt in Field-Grown *Acer* and *Tilia*©

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INTRODUCTION

In 2009 the production area of field-grown ornamental trees in England was estimated to be around 1000 ha, valued at £19.8 million (ADAS, unpublished data). 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 U.K. 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 Jan. 2007, methyl bromide was no longer permitted in the U.K. for pre-plant soil disinfestation for tree production. This led to concern among growers that without an effective alternative the losses incurred to verticillium wilt were likely to increase substantially, effectively preventing the production of certain tree species in the U.K. on a commercial scale. Container production is not a viable option for growing trees to a large size.

Estimating *V. dahliae* levels in field soils in order to give advice on strawberry production has been an established procedure in the U.K. for many years. Based on the results of a soil test, growers are advised on the need for soil disinfestation or the use of wilt-resistant cultivars (Harris et al., 1991). The study reported here aimed to determine a basis for developing strategic planting in two different tree species, *A. platanoides* and *T. cordata*, based on levels of *V. dahliae* in the soil.

METHODS

Creation of a Range of *Verticillium dahliae* Soil Infestation Levels Using Soil Treatments. In 2005, in the first phase of a 5-year 25-plot replicated field experiment, various pre-plant soil treatments were applied to reduce the naturally occurring infestation levels of *V. dahliae* in a field in Hampshire, U.K. The four treatments used were chloropicrin, dazomet + metam sodium, biological soil disinfestations, and a pre-planting cropping with Sudan grass. Untreated plots were also included. These various treatments, together with a natural variation in *V. dahliae* infestation levels across the trial area, resulted in a range of pathogen populations from <0.1 to 62.2 colony forming units cfu/g soil, by January 2006. The results from this aspect of the project have been previously reported (O'Neill et al., 2010).

Regardless of the original treatments, for the purposes of this study, the plots were placed in three groups for statistical analysis. Nine plots had *V. dahliae*

soil infestation levels of less than 0.7 cfu/g soil, six had levels in the range 2.9 to 10.0 cfu/g soil, and nine had levels above 10.0 cfu/g soil (one plot was not treated correctly initially and is disregarded in the analyses). Trees were planted in the Spring 2006 and disease assessments were carried out at the end of the growing seasons in 2006, 2007, and 2008.

Measurement of *Verticillium dahliae* Soil Infestation by Agar Plate Method. Soils from the plots were selected for testing using a wet-sieving method, whereby 25 g air-dried soil was suspended in 50 ml water and agitated for 1 h. The suspension was sieved to retain a 20–160 µm diameter fraction, which was resuspended in 20 ml distilled water. One ml aliquots were spread onto 20 Dox® soil extract plates containing antibiotics and biotin. The plates were incubated for 28 days at 22 °C, and colonies of *V. dahliae* counted after washing soil off the plates (Harris and Yang, 1996).

Assessment of Verticillium Wilt. Trees were assessed each autumn for the occurrence of symptoms potentially due to *V. dahliae* infection. Such symptoms included bark splitting, premature leaf yellowing/necrosis, branch death and, in a few cases, tree death.

A proportion of the *Acer* and *Tilia* trees were destructively sampled from the experiment each year for isolation of *V. dahliae* from a 30-cm section at the base of the main stem. For all stem sections, a 10-cm length was surface-disinfected in sodium hypochlorite (1% available chlorine, 1 min), and the bark was then removed. Three sections (4–6 mm thick) were sawn off, disinfected in alcohol (30 sec), placed onto PDA+streptomycin, and incubated at 22 °C 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 to 21 days for mycelial growth and microsclerotia of *V. dahliae* that had developed from the wood onto the agar.

RESULTS

***Verticillium dahliae* Infestation Density in Soil and Infection in *Acer* and *Tilia*.** No symptoms of verticillium wilt occurred in the first growing season. Field symptoms were first observed in *Acer* in Autumn 2007 and the incidence of both leaf yellowing and bark splitting differed significantly ($P<0.001$) according to soil infestation density with *V. dahliae* (Table 1). There were no obvious external symptoms of infection in the *Tilia* trees at this time, two seasons after planting.

Table 1. Occurrence of bark splits and leaf yellowing with necrosis in *Acer* according to soil levels of *Verticillium dahliae* prior to planting (field assessment, October 2007).

Level of *Verticillium dahliae* (cfu/g) in January 2006	Number of plots	Mean % *Acer* trees with	
		Leaf yellowing + necrosis	Bark split
<0.7	9	8.8	6.9
2.9–10.0	6	26.4	17.9
>10.0	9	32.3	23.9

Isolation from the stem bases in 2006 showed that many *Acer* trees were infected even though no external symptoms had developed (Table 2). There was a trend towards higher levels of infection in *Acer* with increasing soil infestation density of *V. dahliae* in both 2006 and 2007. However, considerable infection was found even in trees from plots with the lowest soil infestation level. Infection in *Acer* was so high by 2008 that no further isolations were undertaken.

In *Tilia*, very little infection had occurred after one growing season (2006) even on plots with very high soil infestation, and no detailed isolations were done that year. In 2007 and 2008 there was a marked increase in infection at the higher soil infestation level (>10 cfu/g) (Table 2 and Fig. 1). The correlation between increasing levels of *V. dahliae* in the soil, on levels of infection in *Tilia,* accounted for 52% and 54% of the variance in 2007 and 2008, respectively.

Table 2. Infection of *Acer* and *Tilia* stem bases according to three soil levels of the fungus at planting.

Verticillium dahliae	Mean % trees infected at stem base (number of growing seasons after planting)			
in soil (cfu/g) at	*Acer*		*Tilia*	
planting in 2006	2006 (1)	2007 (2)	2007 (2)	2008 (3)
<0.7	8.9	16.8	0.0	7.8
2.9–10.0	40.0	47.1	7.8	10.0
>10.0	34.8	37.8	21.7	27.8
Significance	0.009	0.003	0.003	0.003
LSD	21.25	17.31	12.92	12.45

DISCUSSION

The Value of Soil Infestation Threshold Levels for Advisory Purposes. The results from this experiment indicate that the use of a pre-planting test to determine *V. dahliae* levels in soil is not useful for advisory purposes with regard to *A. platanoides* cv. Emerald Queen production, as unacceptable levels of infection may occur even when very low soil infestation (<1 cfu/g) is present. As even the best soil disinfestation treatment is unlikely to eradicate *V. dahliae* in soil, it would be prudent to avoid land with even low levels of infestation, if *Acer* production is planned.

However, there may be scope for using such a test if planting of *Tilia cordata* cv. Greenspire is anticipated. Where *V. dahliae* soil infestation levels were below 0.7 cfu/g soil the pathogen was not isolated from *Tilia* stems after two growing seasons. Low levels of infection were found at that time where the soil level was in the ranges 2.9–10.0 and >10 cfu/g soil and also in the lowest infestation category after three season's growth.

Soil and Other Factors That Might Influence Planting Decisions. A direct relationship between *V. dahliae* inoculum density in soil and disease incidence has been shown in various herbaceous hosts and, less commonly, in trees (Lopez-Escudero and Blanco-Lopez, 2007). In other studies, no relationship could be

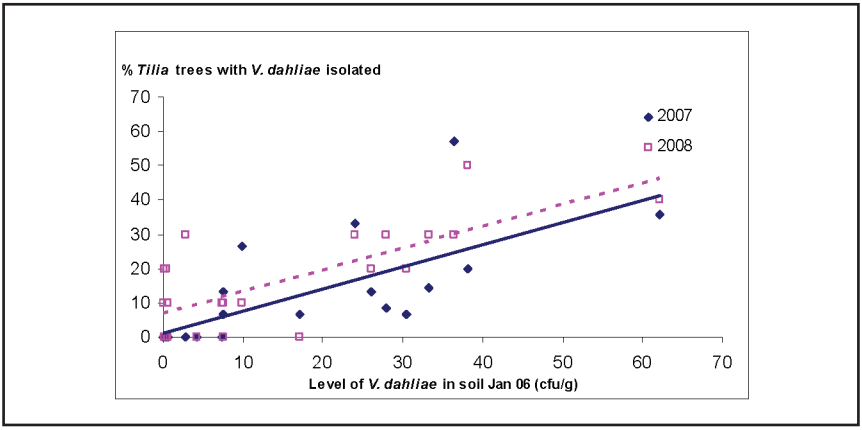

Figure 1. Effect of level of soil infestation with *Verticillium dahliae* at planting on recovery of the fungus from stem bases of *Tilia* after 2 and 3 years.

established, or results were inconsistent and strongly dependent on location. Likely factors which influence infection and make it difficult to develop models that apply generally include *V. dahliae* strain virulence, microsclerotia size and age, soil temperature and moisture, fertiliser use, soil disease suppressiveness, and root growth and architecture (Termorshuizen and Mol, 1995).

The natural ability of some trees to recover from verticillium wilt further complicates the ultimate aim of using soil inoculum density pre-planting to predict disease risk.

Even so, microsclerotial density in soil is a starting point and, as in this work with *Tilia*, many studies show a positive relationship with disease incidence. More data is needed on the factors which influence the relationship between inoculum density and disease progression over the relatively long duration of tree crops such as *Tilia*.

The traditional method for detecting and quantifying *V. dahliae* in soil in the U.K. relies on wet-sieving of soil and plating onto culture medium. Colonies growing from microsclerotia that resemble *V. dahliae* are counted. Other *Verticillium* species which may be found on the plates, mainly *V. tricorpus* and *V. nigrescens*, are disregarded.

Disadvantages of the soil plating method are that it is relatively costly, takes 6 to 8 weeks from sample receipt to reporting and results can vary between laboratories. A molecular, qPCR, test currently in development (Peters et al., 2009) aims to quantify the amount of target pathogen DNA in a few days for a lower cost. Additionally, the molecular test is capable of detecting *V. albo-atrum*, which the conventional test is unable to do.

Work is in progress funded by the Horticulture Development Company (HDC project SF 97) to examine if this new molecular method is able to quantify *V. dahliae* in soil with a sensitivity equal to or better than the plating method and accurately predict risk of verticillium wilt in strawberries, based on a pre-plant soil test.

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