**Contract report for the Horticultural Development Council**

# Tomato: epidemiology and control of *Verticillium* wilt in hydroponic and soil-grown crops

**PC 186a**

**September 2005**

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|---|---|
| Project title: | Tomato: epidemiology and control of *Verticillium* wilt in hydroponic and soil-grown crops |
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| Date project commenced: | 1 October 2003 |
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| Key words: | Tomato, *Verticillium albo-atrum*, wilt, sources, daylength, fungicides |

* The PhD student who worked on this project in year 1 was absent through ill-health during most of year 2 and resigned in August 2005.

The results and conclusions in this report are based on a series of experiments conducted over one year. The conditions under which the experiments were 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**

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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**GROWER SUMMARY**

## Headline

- *Verticillium albo-atrum* was detected by spore-trapping in a tomato crop, indicating aerial transmission of the fungus.
- A non-destructive method of testing for *Verticillium* indicates that there may be many symptomless plants in an affected crop.
- Root drench treatment with carbendazim (Delsene 50 Flo), pyraclostrobin (Comet) and prochloraz (Scotts Octave) all significantly reduced verticillium wilt development. Azoxystrobin (Amistar) was also effective but severely phytotoxic. Only carbendazim treatments (eg Cleancrop Curve, Delsene 50 Flo) are permitted, both at growers own risk under SOLA approvals.

## Background and expected deliverables

HDC project PC 186 (O'Neill & Fletcher, 2002) forewarned UK growers of a new form of verticillium wilt that is a potential serious risk to the economic production of tomatoes. The problem affected at least 10 nurseries in 2001 and was confirmed on a similar number in 2002, affecting crops in England, Wales and Scotland. Previously, a verticillium wilt resistance gene (Ve), incorporated into many commercial European tomato varieties and rootstocks since the late 1970s, had provided good control of verticillium wilt in crops. HDC project PC 186a, in conjunction with Defra project HH3222SPC, is intended as pre-emptive research to devise a solution before the problem becomes more widespread and damaging.

Key points from the 2002 review were:

- The causal fungus is slow-growing and not always easy to find in affected plants.
- Many varieties and rootstocks have been affected.
- The disease is a 'slow wilt' in tomatoes and may be confused with wilting caused by root rotting fungi (e.g. *Pythium*).
- Yield losses were estimated by growers at 10 - 15%.
- The disease is difficult to eradicate from a nursery; it tends to occur in the same glasshouses each year.
- Source of the disease and how it spreads are unknown.
- A carbendazim root drench gives some control if applied early.
- Peppers and cucumbers are also susceptible; two lettuce varieties tested were unaffected.
- No sources of resistance to verticillium, other than the Ve gene, are currently available in either tomato varieties or rootstocks.

This project aims to increase our understanding of the cause and spread of verticillium wilt in tomato crops, of crop cultural and management practices that influence disease expression, and to devise and test practical control measures.

## Summary of the project and main conclusions: Year 2

### Sources of *V. albo-atrum*

Tomato crops on some nurseries and in certain glasshouse become infected by *V. albo-atrum* each year despite apparently thorough nursery hygiene and intensive disinfection between crops. Tests were undertaken to try and identify possible areas where *V. albo-atrum* might persist on a nursery between crops.

Samples of crop debris from the previous season, volunteer tomato seedlings, fallen leaves and fruit, and pathway sweepings collected in Spring 2005 from a nursery with a history of verticillium wilt were examined for *V. albo-atrum* by a very sensitive and specific molecular method (PCR). No *V. albo-atrum* was detected. Method development is in progress to allow testing of water, insects, bees and scrapings from trolley handles and irrigation pegs.

### Spread of *V. albo-atrum*

The current literature indicates that infection by *V. albo-atrum* invariably occurs via the roots. There are no reports of aerial dissemination of spores in tomato crops. Nevertheless, there appears to be potential for aerial spread. Stem lesions bearing sporulating *V. albo-atrum* sometimes develop on tomato plants that have wilted badly or died following infection by the fungus, especially late in the season. Also, sporulation of *V. albo-atrum* has been observed on the undersurface of infected leaves if they are maintained in a humid chamber or laboratory. Season-long spore trapping was therefore undertaken on a nursery in Kent with a history of the disease to examine whether aerial dissemination of the fungus occurs.

Air was aspirated at 10 L/min from a point in the crop 1.5 m above ground level into vaseline-coated slides. The vaseline was removed and tested for *V. albo-atrum* by PCR. *V. albo-atrum* was detected on slides from November 2003 to June 2004, the first detection being in late November, 2 months before verticillium wilt was confirmed in the crop (16 February). These results indicate that aerially-dispersed spores of *V. albo-atrum* may account for outbreaks of the disease and spread within a glasshouse crop. The fungus was not detected on a second nursery in Norfolk where spore trapping was undertaken from March to May following an outbreak of the disease in a few plants in February.

The leaf petiole method developed in year 1 was used to examine the occurrence of symptomless Verticillium within commercial crops. Leaf petioles of some apparently healthy plants of cv. Encore on two nurseries were found to be infected by *V. albo-atrum*. In one location where a few plants at row end were affected by verticillium wilt, 15% of visibly healthy plants along the row were also found to be infected. None of these plants developed obvious symptoms of verticillium wilt in the subsequent 8 weeks.

## Effect of daylength on symptom expression

The effect of daylength on expression of verticillium wilt symptoms was examined by growing inoculated plants, cv. Espero in growth rooms with 8 h and 16 h days. Plants grown in the short daylength first developed wilt symptoms after 2 weeks, whereas plants grown in the long daylength first developed symptoms after 4 weeks. Further tests are required to confirm this result and to determine the effect of natural daylength on verticillium wilt expression in a glasshouse crop.

## Fungicide efficacy

Six fungicide treatments were evaluated for control of verticillium wilt in tomato plants cv. Shirley. The fungicides were applied as a drench to the compost surface before and/or after inoculation with $10^6$ conidia of *V. albo-atrum* in water. Amistar was phytotoxic and plants were severely stunted. Verticillium wilt symptoms (leaf sector yellowing) were seen 28 days after inoculation in all but the Amistar treated plants. By 44 days after inoculation, significantly fewer leaves and plants were infected in the fungicide-treated plants than in the inoculated control. At this time there was significantly better control (% leaves affected) with two carbendazim drenches (Delsene 50 Flo) than with a single application. Infection within stems was confirmed in all of the fungicide treatments.

These results confirm that carbendazim as a drench treatment provides some control of verticillium wilt. The strobilurin fungicides (Amistar and Comet) also gave some control. Amistar controlled symptom expression in leaves but stems were infected and the chemical was severely phytotoxic. Treatment with Amistar at a lower rate warrants investigation, to determine if a rate can be found which is both crop-safe and provides control of the pathogen equal to or better than that of carbendazim.

## Survival of *V. albo-atrum* conidia

Conidia of *V. albo-atrum* were suspended in a droplet of sterile distilled water on a glass slide and the droplets then allowed to dry by air movement from a lamina flow cabinet. The spores were re-suspended in water at increasing time intervals from 5 minutes to 12 hours and tested for viability by plating on to nutrient agar. Wetted conidia survived drying for 6 hours but not for 24 hours. Further work on the effect of air-drying on conidial viability is planned.

## Financial benefits

The UK area of protected tomatoes in 2003 was estimated at 179 ha (4 ha unheated), with a total farm-gate value of around £80.1 million (Defra – Basic Horticultural Statistics for the United Kingdom), Assuming that 5% of the cropped areas suffers a 10% loss due to Verticillium wilt, this equates to £400,425 per annum. Development of an effective strategy to control Verticillium wilt will thus have a significant financial benefit.

**Action points for growers**

1. Plants can be tested for *V. albo-atrum* in a non-destructive manner by testing leaf petioles.

2. Remove severely wilted or dead plants from a crop before sporulation of *V. albo-atrum* develops. Sporulation of *V. albo-atrum* occurs on stem lesions of dying plants, and there is evidence of aerial dissemination of these spores.

3. Consider treatment with an approved carbendazim fungicide (eg Cleancrop Curve, Delsene 50 Flo) if verticillium wilt is confirmed in a crop. Drench treatment of roots with Delsene 50 Flo reduced the development of verticillium wilt. No difference in efficacy was observed between a single drench treatment applied 7 days before inoculation and one applied 7 days after inoculation in a 6-week test.

## SCIENCE SECTION

### 1. Sources of *V. albo-atrum* on a nursery

### Introduction

Plants can be infected in glasshouses by different routes. Possible sources of infection and ways by which *V. albo-atrum* can enter into a glasshouse are:

- Glasshouse soil
- Windblown dust
- Dirt carried by feet, machinery and implements
- Insects
- Airborne spores from crop debris or others sources outside
- Seeds
- Contaminated irrigation water

The objective of this part of the project is to examine these potential sources of *V. albo-atrum* as possible sources of the disease in glasshouse tomato crops.

In 2004, PCR identification confirmed *V. albo-atrum* in fresh tomato stems. A range of other sample types were collected from a nursery where verticillium wilt was present in the crop, and methods have now been developed for testing them as part of the associated Defra project (HH3222SPC).

### Results

The following samples, collected in February and March 2005, were tested for *V. albo-atrum* by PCR and the fungus was not detected:

- dead tomato leaves;
- nursery sweepings (2 sites: Kent and Norfolk);
- volunteer tomato seedlings;
- fallen fruit;
- debris from the previous crop (from outside);
- decomposing trimmings from current crop (under hanging gutter);
- dead tomato stem bases;
- tomato seed collected from a 5 month old crop showing symptoms of Verticillium wilt.

*V. albo-atrum* was detected by PCR at this time in stem sections of visibly affected plants. Method development is continuing, to allow testing of water, insects on sticky traps, bees, scrapings from trolley handles, irrigation pegs, aphids and *Aphidius*.

## 2. Spread of V. albo-atrum

### 2.1 Spore trapping

#### Introduction

Sporulation of *V. albo-atrum* is occasionally seen in commercial tomato crops on stem base lesions of plants infected by the fungus. These lesions may be a source of spores for aerial spread of the pathogen within a tomato crop. At present there is no evidence to support this hypothesis, but conidia of *V. albo-atrum* are produced on diseased lucerne tissue from resting mycelium under cool moist conditions. These conidia can become airborne and have been trapped over lucerne fields. Conidia dispersed by air currents could land on cut lucerne stems resulting in infection. Wilt in lucerne was induced by disseminating conidia in the plant canopy. Rapid secondary spread of verticillium wilt in lucerne was reported to occur following dissemination of spores produced on infected stems, and by contact of these and transported fragments of diseased tissues with wounded surfaces of recently cut lucerne plants. A similar scenario may occur in tomato. Season-long spore trapping on a tomato nursery with a history of verticillium wilt was therefore undertaken.

#### Materials and methods

A Burkard spore trapping machine was installed in tomato nursery in Kent in November 2003, where it is collected air samples by aspirating air at 10 L/min from a point in the crop 1.5 m above ground level. The spores from the air were trapped on vaseline-coated glass slides and tested for *V. albo-atrum* by the polymerase chain reaction (PCR) method, using primers specific for this fungus at the University of Nottingham. A test extraction was carried out using *V. albo-atrum* spores that were added to vaseline-coated glass slides. The method used can detect down to 1,000 spores per glass slide.

Spore trapping was undertaken from November 2003 to October 2004 (year 1) and again from December 2004 to October 2005 (year 2).

Additionally, at a nursery in Norfolk where tomato verticillium wilt was confirmed at a low incidence, spore trapping was undertaken using a Burkard 7-day recording volumetric spore trap from 10 March to 27 April 2005.

#### Results

<u>Kent</u>
The vaseline from slides collected over 2-week periods was bulked and tested. In 2004, *V. albo-atrum* was detected in the vaseline on spore trap slides, by PCR, during the periods November 2003 to February 2004 and May-July 2004 (Figure 2.1 and Table 2.1)). The fungus was not detected in March-April 2004. The identification of verticillium on the slides was confirmed by a second molecular method (T-RFLP).

In 2005, no *V. albo-atrum* was detected during the period December 2004 to May 2005. Results for June-October 2005 are still being determined.

Norfolk

No *V. albo-atrum* was detected on spore trap slides.

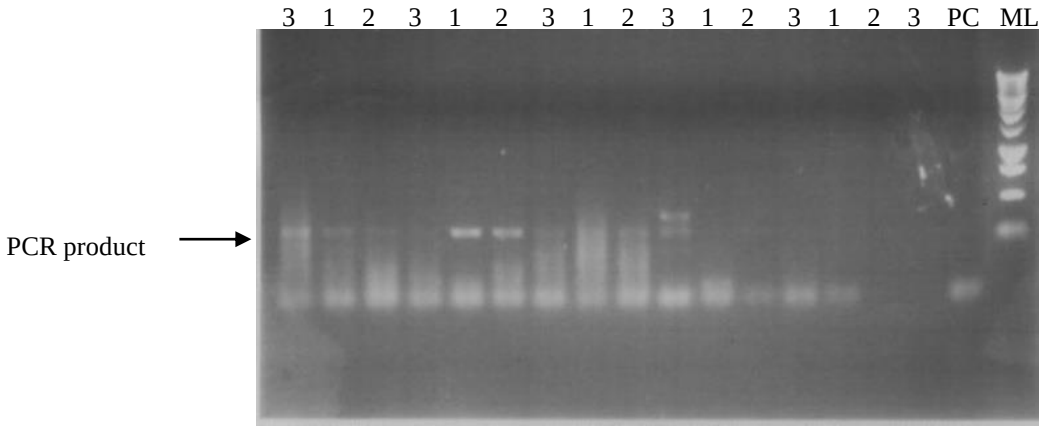



**Figure 2.1** PCR results for DNA extracted from air samples collected from November 2003 until March 2004. 1, 2, 3 represent pooled samples collected from dates: 1st-10th, 11th-20th and 21st till end of the month respectively. PC- Primer control, ML- Mass Ladder. A white band opposite the arrow indicates occurrence of *V. albo-atrum* in the sample.

**Table 2.1** Detection of *V. albo-atrum* in vaseline extracts from spore trapping slides on a nursery in Kent

| | ***V. albo-atrum* detected** | | |
|---|---|---|---|
| Month | 2003 | 2004 | 2005 |
| January | NT | + | - |
| February | NT | + | - |
| March | NT | - | - |
| April | NT | - | - |
| May | NT | + | - |
| June | NT | + | NT |
| July | NT | + | NT |
| August | NT | - | NT |
| September | NT | - | NT |
| October | NT | - | NT |
| November | + | - | NT |
| December | + | - | NT |

+ *Vaa* detected, - Samples tested and no *V. albo-atrum* found; NT-not tested

**Discussion**

These results are the first report indicating aerial dissemination of *V. albo-atrum* in a tomato crop. The origin of *V. albo-atrum* detected on spore traps (presumably as conidia) is unknown. There were no stem base lesions with sporulation of *V. albo-atrum* visible in the crop at that time the fungus was first detected in the air (late November). Slight wilting in the crop was first observed at the end of January and *V. albo-atrum* was first confirmed in the crop on 16 February.

## 2.2 Disease monitoring on nurseries by testing leaf petioles

**Introduction**

In 2004, testing of leaf petioles proved to be an effective non-destructive method for determining occurrence of verticillium wilt in a crop. Petiole sections were incubated for 7-14 days and then examined microscopically for verticillate sporulation. In 2005, further testing was undertaken and some sets were tested for *V. albo-atrum* by PCR.

**Methods**

Samples of lower leaves were collected at random, 1 leaf/plant, from visually healthy plants in glasshouse blocks with a history of verticillium wilt (site A, Kent) or with a low incidence of wilt (site B, Norfolk). Ten transverse sections were examined from each petiole. The crop at site A was ungrafted cv. Encore, grown on rockwool slabs on the floor in a glasshouse where the disease had occurred in each of the last 3 years. The crop at site B was ungrafted cv. Encore grown on rockwool slabs on hanging gutters, in a glasshouse where there had been no history of the disease in previous seasons.

**Results**

Site A

Plants were received in December 2004 and planted in January 2005. Carbendazim drench treatments were applied on 14 January, 17 March, 11 June and 31 July. Verticillium wilt was not obvious on the nursery this year and yields were better than in previous years. Stem botrytis was widespread by September.

Results of petiole tests on six dates from March to September were as follows:

| | |
|---|---|
| 23 March: | Block B: 57 plants tested, 0 positive |
| 16 May: | Block B: 53 plants tested, 1 positive |
| 4 July: | Block B: 55 plants tested, 3 positive |
| 8 August: | Block B: 54 plants tested, 27 positive |
| 19 September: | Block B: 60 plants tested, 21 positive. |

Site B

April 2005

Verticillium wilt had been confirmed on the nursery in a few plants at the end of one row (row 76 East) in February 2005. In early April, one lower leaf was collected from each of 40 visibly healthy plants along this row, by taking 20 leaves from each side of the row. Verticillium was confirmed in 2 of 20 leaves on the one side of the row and in 3 of 20 leaves on the opposite side. In three of the petioles, verticillium was confirmed in 9 of 10 or 10 of 10 petiole sections.

May 2005

Ten petioles were collected from each of 42 rows. Each of 5 rows either side of the known infected area were sampled, together with a further 22 rows spread evenly throughout the rest of the variety in the block (approximately one row in 10). Samples from each row were bulked for testing. *V. albo-atrum* was confirmed in two of the 42 rows sampled, rows 55 East and 110 West, both distant from the known infected row (76 East). No symptoms of verticillium wilt were observed in rows 55 East and 110 West during the rest of the season (to early September 2005).

June 2005

The 42 rows sampled in May were re-tested. No *V. albo-atrum* was detected. The samples are being re-tested.

**Discussion**

The results from both sites confirm those of 2004, in that verticillium wilt can be present within a crop at a much greater level than is apparent from visible symptoms of the disease.

## 3. Effect of daylength on symptom expression

### Introduction

The objective of this study was to find out how daylength influences symptom development in verticillium (Ve)-resistant tomatoes, and if symptoms differ in short and long daylength conditions.

### Methods

Inoculation
Spore suspensions were prepared from 14-day-old culture of *V. albo-atrum* (isolate AR01/036) was grown on PDA at 20-22°C and a sporulating culture washed with 1 ml of sterile distilled water. The concentration was adjusted to $10^6$ and $10^4$ spores/ml and 10 plants were used as a control inoculated with water.

Plant production
Tomato plants cv. Espero F1 were grown in small pots (45 $cm^3$) before transfer to larger (1 L) pots at the 2-true-leaf stage and inoculated with 1 ml of the fungal spore suspension into the root area using a Gilson Pipette. Plants were grown in the growth room with a 16-hour day length and at temperatures of 20°C day and 18°C night. Five weeks after inoculation the experiment was stopped. The growth room was switched to an 8-hour day length and a second set of plants at the 2-true leaf stage were introduced and inoculated.

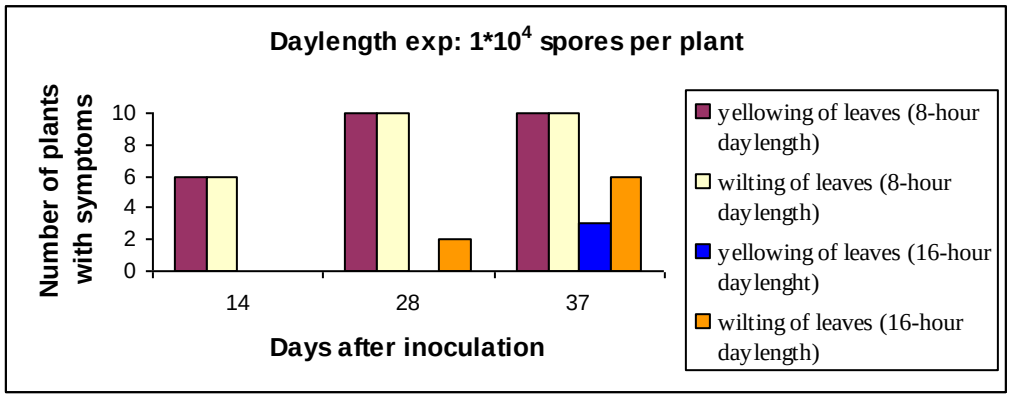
For both the 16-hour and 8-hour treatments, 10 plants were inoculated with a concentration of $10^6$ spores/ml, 10 with a concentration of $10^4$ spores/ml and 10 with sterile distilled water (SDW), as a control.

Statistical analysis
Standard errors (SE) were calculated for the numbers of plants with symptoms. SEs were calculated as $\sqrt{n \times p \times q}$ where n is the total number of plants, p is the proportion with symptoms and q=1-p.

### Results and discussion

As can be seen from the Figure 3.1, at both concentrations of the verticillium inoculum, plants developed wilt symptoms. Tomato plants grown in the short days showed symptoms 2-weeks after inoculation. However, plants grown in long days developed first symptoms after 4 weeks.

This initial experiment indicates that plants in a growth room inoculated with conidia of *V. albo-atrum* by a root drench treatment develop symptoms of verticillium wilt significantly more quickly when grown in short days (8 h light) than long days (16 h light). Further tests are required to confirm this result, and to determine the effect of natural daylength on verticillium wilt expression in a glasshouse crop.



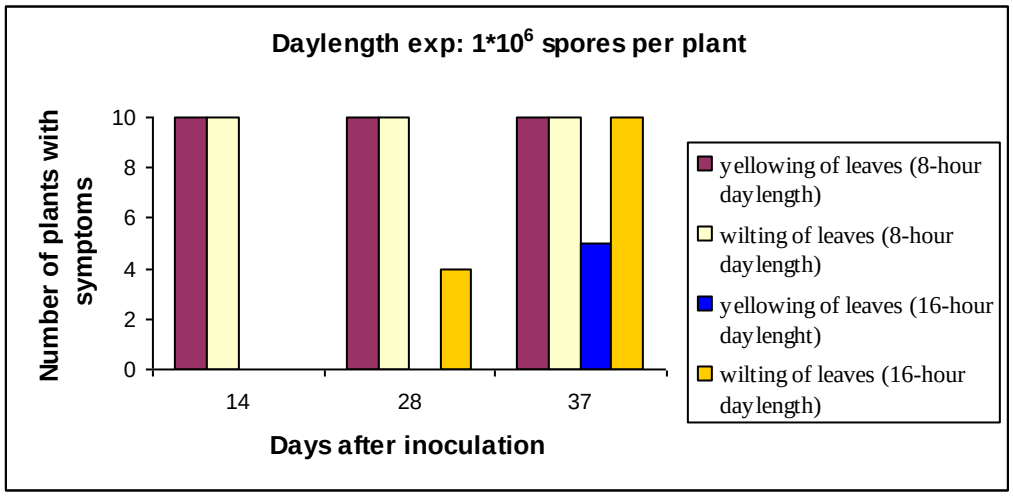




**Figure 3.1** Effect of day-length and inoculation treatment on verticillium wilt symptom development

**Table 3.1** Effect of day length on verticillium wilt symptom developments – leaf yellowing.

| Treatment | No plants (of 10) with leaf yellowing after | | |
|---|---|---|---|
| | 14 | 28 | 37 days |
| Low inoculum ($10^4$ conidia) | | | |
| 1. 8 h day | 6 (1.55) | 10 | 10 |
| 2. 16 h day | 0 | 0 | 2 (1.26) |
| High inoculum ($10^6$ conidia) | | | |
| 3. 8 h day | 10 | 10 | 10 |
| 4. 16 h day | 0 | 0 | 5 (1.58) |

( ) – standard errors

**Table 3.2**. Effect of daylength on verticillium wilt symptom development – leaf wilting.

| Treatment | No plants (of 10) with leaf wilting after | | |
|---|---|---|---|
| | 14 | 28 | 37 days |
| Low inoculum ($10^4$ conidia) | | | |
| 1. 8 h day | 6 (1.55) | 10 | 10 |
| 2. 16 h day | 0 | 2 (1.26) | 6 (1.55) |
| High inoculum ($10^6$ conidia) | | | |
| 3. 8 h day | 10 | 10 | 10 |
| 4. 16 h day | 0 | 4 (1.55) | 10 |

( ) – standard errors

## 4. Evaluation of fungicide treatment

### Introduction

Currently the only fungicide permitted for treatment of verticillium wilt is carbendazim, which is applied as a drench to the roots. Three products (Bavisitin DF, Cleancrop Curve and Delsene 50 Flo) are approved under the SOLA scheme. The efficacy of this treatment is uncertain. Further, some retailers do not permit its use until after the disease has been confirmed. An experiment was therefore devised to investigate the efficacy of four fungicides for control of verticillium wilt. Carbendazim was evaluated both as a preventative and a curative treatment.

### Objectives

To determine the efficacy of six fungicide treatments applied as root drenches for control of *V. albo-atrum* in protected tomato cv. Shirley.

### Experimental design and statistical analysis

There were a total of eight treatments, with ten tomato plants per plot, and four replicate blocks. Observation plots containing three plants each were given fungicide treatment, but not inoculated, to assist in distinguishing any phytotoxic effects from symptoms caused by verticillium wilt.

The results were analysed either by ANOVA, or by regression analysis, as appropriate. ANOVA was performed on the number of plants and leaves affected, the maximum height to which leaves were affected (measured as the number of leaves affected, counted upwards from the plant base). Regression analysis was used for the number of leaves affected and the total leaves, to give the proportion of leaves affected, so that greater statistical weight was given to plants with a larger number of leaves. ANOVA was carried out on the number of fruit trusses, the proportion of the circle of xylem band around the stem affected (as a percentage) at 0 cm and 20 cm from the stem base, and the number of plants affected at these heights.

### Treatments

Six treatments (treatments 3-8) received both inoculation and fungicide application. Two treatments (treatments 1-2) did not receive fungicide application and of these one (treatment 1) did not receive inoculation. Details of individual treatments are given below.

| | Fungicide | Drench timing |
|---|---|---|
| **1.** | Uninoculated | Untreated |
| **2.** | Inoculated | Untreated |
| **3.** | Delsene 50 Flo | 7 days before inoculation, |
| | (500 g/L carbendazim) | at 0.5 ml/litre |
| **4.** | Delsene 50 Flo | 7 days after inoculation, |
| | (500 g/L carbendazim) | at 0.5 ml/litre |
| **5.** | Delsene 50 Flo | 7 days before and after inoculation, |
| | (500 g/L carbendazim) | at 0.5 ml/litre for each application |
| **6.** | Amistar | 7 days before and after inoculation, |
| | (250 g/L azoxystrobin) | at 1.0 ml/litre for each application |
| **7.** | Comet | 7 days before and after inoculation, |
| | (250 g/L pyraclostrobin) | at 1.0 ml/litre for each application |
| **8.** | Scotts Octave | 7 days before and after inoculation, |
| | (46% w/w prochloraz) | at 0.5g/litre for each application |

**Methods**

Spore inoculation

Plants were inoculated with a drench of 1 ml of (isolates AR01/36 plus AR05/15) at 1 x $10^6$ conidia/ml in 100 ml water. Plants were inoculated 4 weeks after sowing when they had 4-5 true leaves.

Using an inoculation loop, verticillium conidia from a 14-day-old culture were spread over plates of PDA+streptomycin. Plates were incubated in the dark at 25ºC for 6 days. Verticillium conidia were collected by washing the plate with sterile distilled water. This spore suspension was filtered through muslin to remove any mycelial strands. The spore concentration was adjusted to $10^6$ conidia / ml.

1 ml of this $10^6$ conidia / ml spore suspension was applied as a drench to the roots, applied around the stem base in 100 ml of water to allow the moist compost to become wetted throughout to the point of run-through.

Crop production

Plants were grown on for 7 weeks after inoculation. Tomato plants in rockwool plugs at the 2-3 true-leaf growth stage were potted on into 13 cm diameter pots containing F1 compost. For each treatment, pots were placed onto a gravel tray lined with capillary

matting to aid daily watering and to prevent any cross-infection between pots by drainage water. A gap of at least 30 cm was allowed between adjacent plots.

The glasshouse temperature was set at 18ºC day and 16ºC night with the maximum vent set at 28ºC. Compost and air temperatures were recorded electronically. From 16 days after potting, plants were watered with potassium nitrate. A stock solution of 150 g nitrate was prepared in 1 L water. This was diluted 1 in 200 before feeding the crop to give 105 mg/l nitrogen and 340 mg/l potassium. From 35 days after potting, and on a weekly basis, tomato plants were twisted around supporting strings to maintain stem support. Side shoots and fruit were removed as necessary.

Disease severity

Each plant was assessed at 28 days and 44 days after inoculation for % leaf area affected, the number of leaves affected by wilting or yellowing, and the maximum height above the stem base to which externally visible verticillium symptoms extended. The number of plants affected was recorded for each treatment. The growth stage of each plant (maximum number of leaves) was noted. The number of fruit trusses produced by each plant was recorded at day 28. At day 41 the height of a sample of plants within each plot was measured.

Stem colonisation

Destructive sampling was commenced 47 days after inoculation. Each pot was sampled separately. Lengths of stem, 3 cm long, were cut from each plant, using secateurs, at 20 cm intervals starting at the base of the plant and continuing to a height of 1 m. Each of the six lengths per plant were labelled with their original position.

Each stem piece was dipped in 70 % ethanol and allowed to dry, followed by 2 minutes in 0.5 % solution of sodium hypochlorite. The central 5 mm piece from each 3 cm stem piece was cut and placed onto damp filter paper in a Petri dish, so that the stem position of samples testing positive for verticillium could be identified. All Petri dishes were incubated on the bench surface at approximately 20ºC for 16 days. Each stem piece was examined for the presence of verticillium within any part of the circle of the xylem band.

**Results**

Incidence and severity of verticillium wilt

Angular areas of leaf yellowing, typical of verticillium wilt, were observed at 28 days after inoculation (DAI) on a small proportion of inoculated plants in most treatments (Table 4.1).

Treatments differed significantly in the mean numbers of plants affected, numbers of leaves affected and height of wilt symptoms at 44 DAI, although not at 28 DAI (Table 4.1). Treatments differed significantly in % leaves affected (Table 4.2 and regression table) at both assessment dates. At 28 DAI the percentage of leaves affected was reduced from 4.95% in the inoculated control to less than 1.5% following the fungicide treatments. At the 28 day assessment, but not at the 44 day assessment, there was

evidence of slightly greater control from carbendazim applied as a preventative application compared with treatment 7 days after inoculation. At 44 DAI, carbendazim applied twice gave significantly better control than a single application either pre- or post-inoculation. At this time, the mean maximum height of leaves with symptoms (determined by counting upwards from the stem base) was 2.0 on inoculated plants, and less than 1.0 for all fungicide treatments. Amistar was the best fungicide treatment with no external symptoms of verticillium wilt in treated plants; however, the stems were found to be infected (see below), and treatment was phytotoxic (see below).

Stem colonisation

The number of plants affected and the proportion of the circle of the xylem band around the stem that developed verticillium were significantly greater in the inoculated untreated plants at both 0 cm and 20 cm up the stem compared with all other treatments (Table 4.3). Delsene 50 Flo applied 7 days post-inoculation had the least number of stems affected, and the least vascular bundle colonisation, at both 0 and 20 cm height. Stem colonisation above 20 cm was not analysed because of the sparse occurrence of positive values.

Crop growth

Amistar was phytotoxic and by seven days after the first drench the growing tips were damaged. The first two leaves were normal, but the third and fourth were half the normal size, and the expanding fifth leaf was either shrivelled or was severely stunted. After the second drench application, some growing points were completely destroyed and either several side shoots were produced from just above the cotyledons or a shoot was produced from just below the damaged apex. By 28 DAI, the leaves showed regular patterning in both the inoculated experiment and in the three observation plants treated with Amistar but not inoculated. Analyses included measurements on side shoots where the main shoot had been killed, and plants with lower leaf numbers were included to show the effect of the phytotoxicity. Treated plants had a significantly shorter mean height (Table 4.4). The mean number of trusses produced for Amistar treated plants was also significantly less than the rest. No other fungicide produced phytotoxic effects.

**Discussion**

These results confirm that carbendazim as a drench treatment provides some control of verticillium wilt. By 44 DAI, there was significantly better control (of % leaves affected) from a double carbendazim treatment compared with a single application. The strobilurin fungicides (Amistar and Comet) also gave some control. Although Amistar appeared particularly effective at the rate used, it controlled foliar symptoms without reducing stem infection. There is no approval for use of Amistar as a drench treatment on tomato and use would be precluded because of its marked phytotoxicity. Treatment with Amistar at a lower rate warrants investigation, to determine if a rate can be found which is both crop-safe and provides control of the pathogen that is equal to equal to, or better than, that of carbendazim.

Leaf wilting was not observed at either 28 DAI or 44 DAI. This was probably because the plants were not sufficiently water stressed, as the assessment period fell during a period

of cool, overcast weather that gave lower glasshouse temperatures.

**Table 4.1.** Effect of fungicide treatment on mean number of plants affected, mean number of leaves affected and maximum height up the stem of leaves with verticillium wilt symptoms (leaf number from stem base).

| Treatment | Mean no plants (of 10) with *Vaa* wilt symptoms | | Mean no leaves/plants with wilt symptoms | | Mean maximum height (leaf number) with foliar symptoms | |
|---|---|---|---|---|---|---|
| | 28 DAI | 44 DAI | 28 DAI | 44 DAI | 28 DAI | 44 DAI |
| 1. Uninoculated control | 0 | 0.1 | 0 | 0.08 | 0 | 0.20 |
| 2. Inoculated control | 0.3 | 0.5 | 0.78 | 1.33 | 1.00 | 2.03 |
| 3. Delsene 50 Flo (7d pre) | 0.1 | 0.3 | 0.08 | 0.38 | 0.18 | 0.93 |
| 4. Delsene 50 Flo (7d post) | 0.2 | 0.2 | 0.23 | 0.25 | 0.55 | 0.53 |
| 5. Delsene 50 Flo (x2) | 0.1 | 0.1 | 0.15 | 0.08 | 0.43 | 0.20 |
| 6. Amistar (x2) | 0 | 0 | 0 | 0 | 0 | 0 |
| 7. Comet (x2) | 0.1 | 0.1 | 0.15 | 0.10 | 0.20 | 0.15 |
| 8. Scotts Octave | 0.1 | 0.2 | 0.10 | 0.25 | 0.18 | 0.40 |
| Df | 21 | 21 | 21 | 21 | 21 | 21 |
| SED | 0.12 | 0.104 | 0.302 | 0.274 | 0.359 | 0.376 |
| Significance | 0.258 | 0.002 | 0.266 | 0.002 | 0.153 | <0.001 |

(x2) - treatments were applied twice, 7d pre-inoculation and 7d post-inoculation.
*Vaa* - *V. albo-atrum*; DAI- days after inoculation

**Table 4.2**. Effect of fungicide treatment on % leaves affected by symptoms of verticillium wilt.

| Treatment | % leaves affected (± SE)* | |
|---|---|---|
| | 28 DAI | 44 DAI |
| 1. Uninoculated control | 0.00 (0.002) | 0.37 (0.181) |
| 2. Inoculated control | 4.95 (0.562) | 6.73 (0.743) |
| 3. Delsene 50 Flo (7d pre) | 0.49 (0.190) | 1.91 (0.411) |
| 4. Delsene 50 Flo (7d post) | 1.46 (0.322) | 1.27 (0.336) |
| 5. Delsene 50 Flo (x2) | 0.98 (0.266) | 0.39 (0.194) |
| 6. Amistar (x2) | 0.00 (0.002) | 0.00 (0.004) |
| 7. Comet (x2) | 1.01 (0.273) | 0.52 (0.218) |
| 8. Scotts Octave (x2) | 0.64 (0.214) | 1.30 (0.345) |

* predicted from regression analysis.
(x2) - treatments were applied twice, 7d pre-inoculation and 7d post-inoculation.
DAI- days after inoculation

Regression analysis of % leaves affected by symptoms of Verticillium wilt.

| | | 28 DAI | | 44 DAI | |
|---|---|---|---|---|---|
| Change | Df | Mean deviance | F prob. | Mean deviance | F prob. |
| + Block | 3 | 24.74 | <0.001 | 13.81 | <0.001 |
| + Treatment | 7 | 10.89 | <0.001 | 17.94 | <0.001 |
| Residual | 308 | 0.45 | | 0.71 | |
| Total | 318 | 0.91 | | 1.21 | |

**Table 4.3.** Effect of fungicide treatment on colonisation of stems by *V. albo-atrum* at 44 days after inoculation

| Treatment | Mean no stems affected (of 10): | | % vascular bundle affected: | |
|---|---|---|---|---|
| | At stem base | 20 cm up the stem | At stem base | 20 cm up stem |
| 1. Uninoculated control | 2.3 | 0.5 | 15.2 | 1.4 |
| 2. Inoculated control | 6.5 | 5.3 | 63.0 | 40.3 |
| 3. Delsene 50 Flo (7d pre) | 2.8 | 1.3 | 18.7 | 10.6 |
| 4. Delsene 50 Flo (7d post) | 0.8 | 0 | 4.6 | 0 |
| 5. Delsene 50 Flo (x2) | 2.3 | 0.5 | 16.0 | 1.9 |
| 6. Amistar (x2) | 2.3 | 1.0 | 16.4 | 2.9 |
| 7. Comet (x2) | 1.3 | 0.5 | 6.7 | 3.8 |
| 8. Scotts Octave | 2.3 | 1.5 | 16.7 | 6.5 |
| | | | | |
| Df | 21 | 21 | 21 | 21 |
| SED | 1.14 | 1.36 | 10.96 | 11.39 |
| Significance | 0.003 | 0.024 | 0.001 | 0.036 |

(x2) - treatments were applied twice, 7d pre-inoculation and 7d post-inoculation.

**Table 4.4**. Effect of fungicide treatment on plant growth

| Treatment | Mean no fruit trusses (28 DAI) | Mean plant height (m) at day (44 DAI) |
|---|---|---|
| 1. Uninoculated control | 1.83 | 1.35 |
| 2. Inoculated control | 1.98 | 1.38 |
| 3. Delsene 50 Flo (7d pre) | 1.73 | 1.36 |
| 4. Delsene 50 Flo (7d post) | 1.95 | 1.39 |
| 5. Delsene 50 Flo (x2) | 1.98 | 1.40 |
| 6. Amistar (x2) | 1.18 | 1.14 |
| 7. Comet (x2) | 1.82 | 1.35 |
| 8. Scotts Octave | 1.93 | 1.40 |
| | | |
| Df | 21 | 21 |
| SED | 0.123 | 0.054 |
| Significance | <0.001 | 0.002 |

(x2) - treatments were applied twice, 7d pre-inoculation and 7d post-inoculation.
DAI- days after inoculation

## 5. Survival of *V. albo-atrum* conidia

### Introduction

An experiment was devised to determine the effect of air-drying for up to 24h on viability of *V. albo-atrum* conidia.

### Treatments

1. Nil drying
2. Air-drying for 5 mins
3. Air-drying for 30 mins
4. Air-drying for 1h
5. Air-drying for 3h
6. Air drying for 6h
7. Air-drying for 24h
8. Suspended in water for 24h (control)

### Method

A suspension of *V. albo-atrum* in sterile distilled water (SDW) was prepared from a young sporulating culture, around $10^5$ spores/ml. Three crosses were marked on one side of new glass slides. One 10 µl droplet of recently-agitated spore suspension was placed on the reverse side of the slide in the centre of each cross. There were 3 replicate slides (9 spots in total) for each time period. Droplets were allowed to dry in the laboratory in a laminar flow cabinet (around 20 mins). After the given dry-time period had elapsed for each treatment (5 mins to 24 h), a 10 µl droplet of SDW was carefully placed onto each area of dried spores, left for 5 minutes, then pipetted up and spotted onto a PDA + S plate. A new pipette tip was used between each inoculation when sucking up re-suspended spores. At time zero (treatment 1) and after 24 h (treatment 8), 9 x 10 µl droplets of the spore suspension were pipetted directly onto a plate, in a 3 x 3 array The spore suspension remained on the laboratory bench adjacent to the inoculated slides for this period. PDA + S plates were incubated at 20°C and the number of inoculated areas (of 9) showing any verticillium growth was assessed after 7 and 14 days.

### Results

**Table 5.1.** Effect of air drying on viability of *V. albo-atrum* conidia

| Number of droplets (of 9) retaining viable *V. albo-atrum* after air drying for: | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0 mins | 5 mins | 30 mins | 1 h | 3 h | 6 h | 24 h | Control (suspended in water for 24 h) |
| 9 | 9 | 9 | 9 | 6 | 6 | 0 | 9 |

This experiment indicates that wetted conidia can survive air-drying for 6 but not 24 h. The effect of drying on the viability of *V. albo-atrum* condidia will be investigated further in year 3.

## 6. Overall conclusions

1. No source of *V. albo-atrum* which permits carry over of the pathogen between crops has been identified. No *V. albo-atrum* was detected in samples of leaf debris, nursery sweepings, volunteer seedlings or fallen fruit taken in February-March 2005 from two glasshouse blocks with verticillium wilt present, one with a history of the disease over several years.

2. *V. albo-atrum* can occur in the air within a crop. The fungus was detected on a spore trap in a crop from November 2003 to February 2004, and again later in the season, the first detection being before symptoms of verticillium wilt were apparent in the crop (end of January).

3. This work confirmed that many symptomless plants might occur in a crop affected by verticillium wilt.

4. An initial experiment indicates that inoculated plants in a growth room develop symptoms of verticillium wilt more quickly when grown in short days (8 h light) than long days (16 h light). Further tests are required to confirm this result and to determine the effect of natural daylength on verticillium wilt expression in a glasshouse crop.

5. The incidence and severity of verticillium wilt was significantly reduced by drench treatment with carbendazim (Delsene 50 Flo), prochloraz (Scotts Octave) and pyraclostrobin (Comet). Azoxystrobin (Amistar) drench greatly reduced foliar symptoms but was severely phytotoxic.

6. In a single experiment, wet conidia of *V. albo-atrum* withstood air-drying for 6 h but not 24 h.

## 7. Technology transfer

Meetings

1. Project review meeting, Warwick-HRI, 26 January 2005.
2. Project review meeting, University of Nottingham, 15 July 2005.

Articles

O'Neill TM. New results on tomato verticillium wilt. *HDC News* **113**, 21-23.

O'Neill TM. Understanding verticillium wilt in glasshouse tomatoes. *Plant It!* **7**, p6.

Poster

Krishnamurthy V, Rossall S & O'Neill TM. Detection and suppression of *Verticillium albo-atrum* aggressive on Ve-resistant tomato cultivars. *Society of General microbiology*, Keele University, 12 September 2005.

## 8. **Acknowledgements**

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